CHARACTERISATION & BSA REMOVAL OF TACC3 ANTIBODY VIA IMMUNOFLUORESCENCE & IMAGING MASS CYTOMETRY

Shakila Khalifehzadeh*, Lexy Sarginson, Dr Ben Wetherall & Dr Suzanne Madgwick
200116963; s.khalifehzadeh2@ncl.ac.uk

Introduction

- Cell division in human eggs is highly error prone, and therefore eggs with abnormal chromosome numbers are commonly produced. This is a major contributor to miscarriage and developmental disabilities such as Down syndrome [1, 2].
- Currently we lack an overall picture of the regulation of key proteins involved in division and how they vary in amount and location relative to one another.
- Analysing eggs by imaging mass cytometry (IMC) is an ideal solution. This cutting-edge technique can detect up to 40 proteins of interest simultaneously using antibodies tagged with metal isotopes [3].
- For IMC, successful tagging requires antibodies to be free from bovine serum albumin (BSA) as it competes with the primary antibody for the desired label which significantly reduces efficiency [4].
- TACC3 has shown a role in preserving spindle stability; direct knock-down of the protein causes abnormal spindle development [5].
- Its significant role has also been demonstrated by the genetic deletion of TACC3 in mice, which leads to death during mid-late gestation [5].
- Considering the protein's key role, we hypothesised this would be a good antibody to trial BSA removal and determine the protein's characteristics throughout egg cell division via IMC.

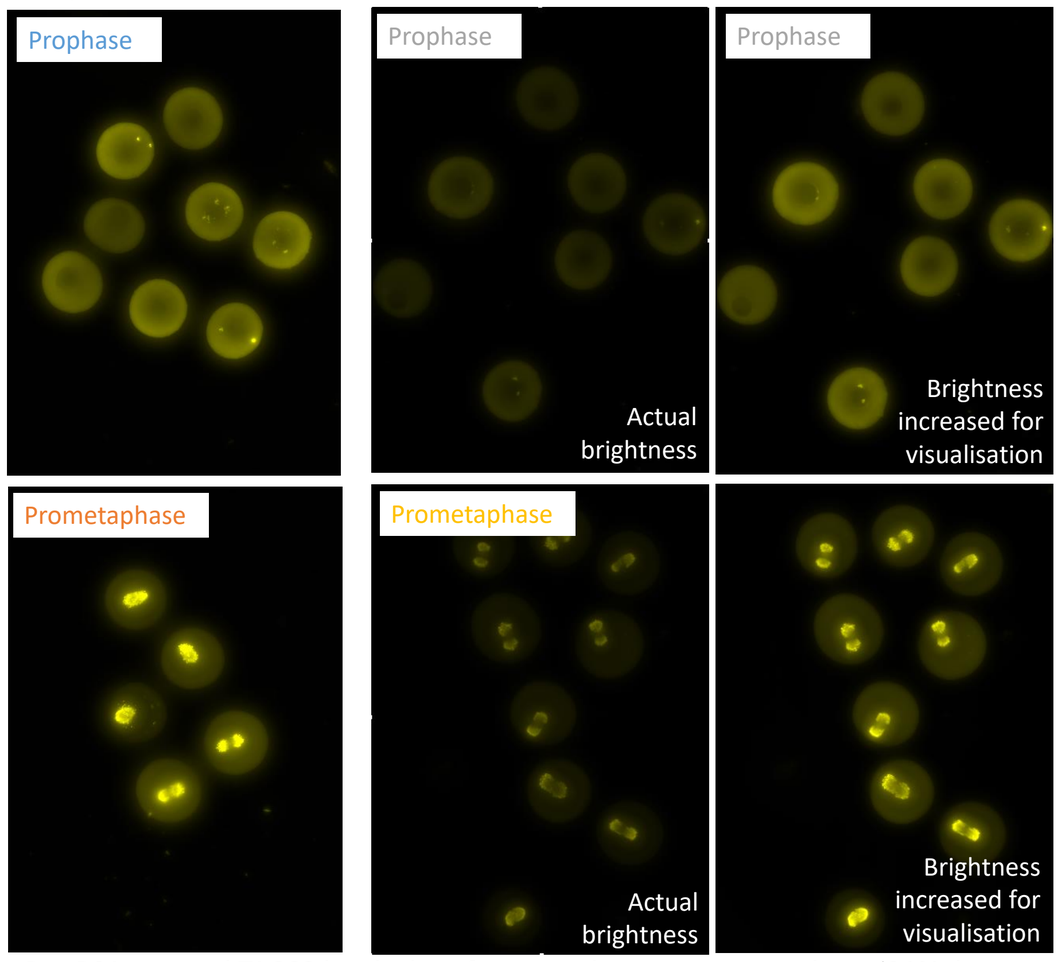
Aims

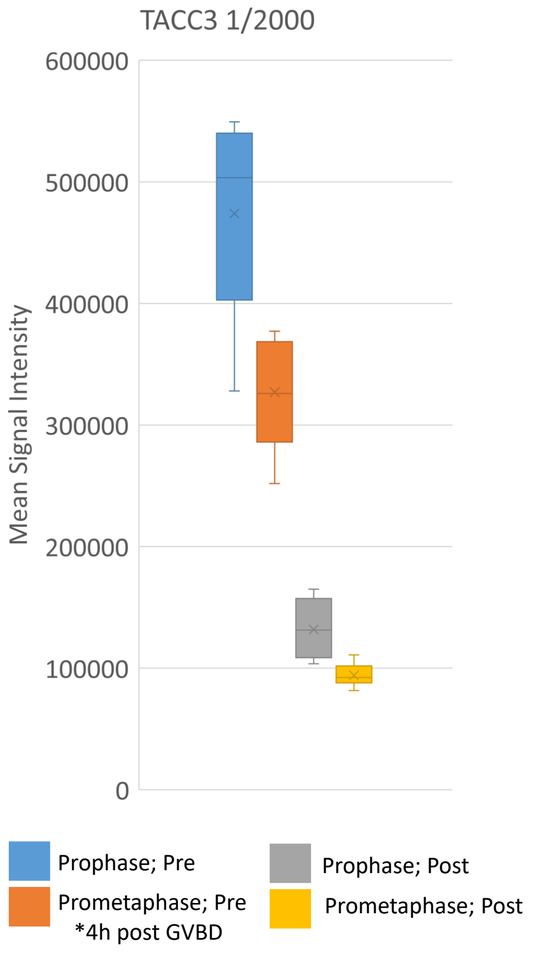
1. To characterise TACC3 behaviour throughout egg cell division
2. To trial antibody BSA removal prior to metal isotope tagging

Methods

Egg selection and fix → Immuno-fluorescence (IF) to validate TACC3 antibody → TACC3 antibody BSA removal → TACC3 antibody conjugated to metal tag → Immuno-fluorescence to revalidate TACC3 following conjugation → IMC to determine antibody capabilities

Immunofluorescence validation pre and post BSA removal:

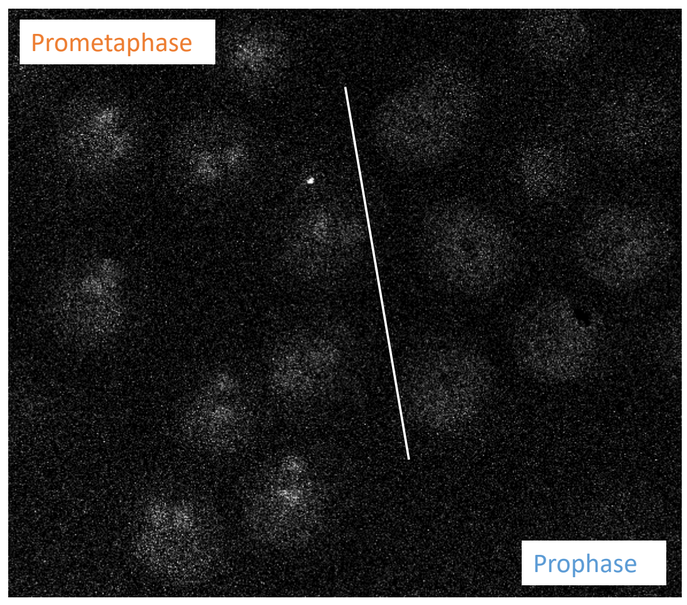


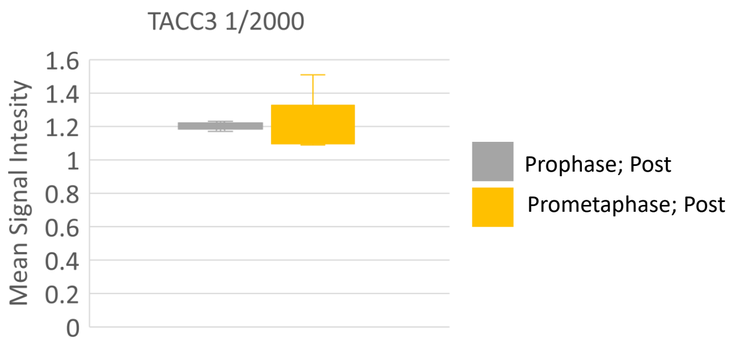


- Pre-BSA removal TACC3 levels drop from prophase to prometaphase (31% decrease), changes in localisation are also observed, in prophase the protein is widespread throughout the cell with the exception of the nucleus and then moves to the spindle in prometaphase.
- Reassuringly the protein localises in the same way following BSA removal (clearly seen in the images with increased brightness) and also decreases by a similar amount from prophase to prometaphase (29%)
- Understandably there is some loss in protein density following BSA removal, but the strength of the antibody still clearly shows protein characteristics

*GVBD = germinal vesicle breakdown, which is the breakdown of the nucleus signalling the next stage of division

IMC of TACC3:



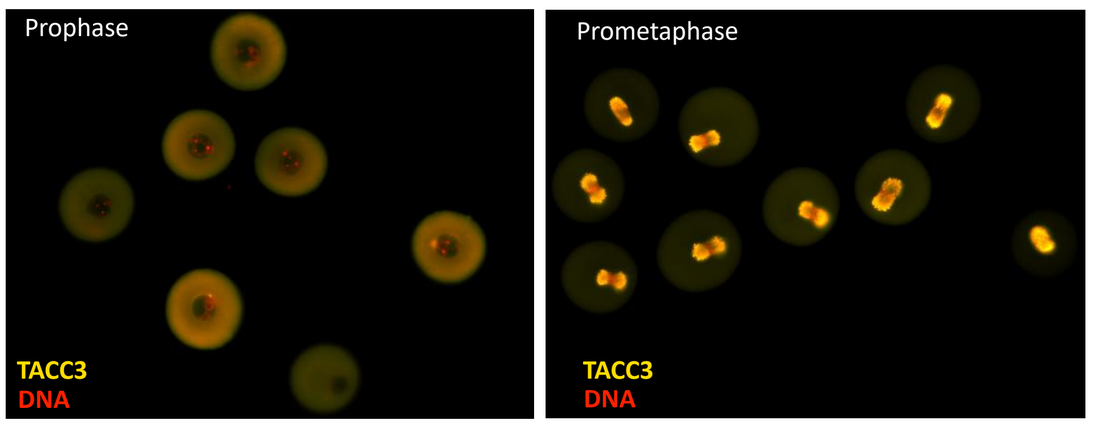


Although the work needs optimising, we can see expected localisation of TACC3.

- IMC shows the protein throughout the cell in prophase in the absence of the nucleus, and at the spindle in prometaphase
- Protein levels do not decrease as shown via immunofluorescence, but we expect to see this in future

Key Finding

We have observed (both pre and post BSA removal via IF and IMC) that the localisation of TACC3 changes from being widespread throughout the cell (barring the nucleus) in prophase to the spindle in prometaphase. This is expected based on its role in spindle assembly.
Signal intensity did however decrease following BSA removal, which is to be expected based on method inefficiencies.



Conclusion & Future Work

The TACC3 antibody was characterised by both IF and IMC, which shows a change in localisation; this will be a useful protein when trying to identify faulty eggs via IMC. The success of BSA removal is likely heavily dependent on the original strength of the antibody due to method inefficiencies of BSA removal and tagging of isotopes.
Future work will be to optimise the TACC3 antibody, trying different concentrations via IMC.

References

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