Natalie Perrett
Email:
N.F.Perrett1@Newcastle.ac.uk
Mobile: 07939228176
Faculty of Biomedical Science

Chromosome choreography by SUN1-KASH5 in mammalian meiosis

With thanks to Dr Owen Davies, Dr Orla Dunne, James Dunce, Lucy Salmon, Matthew Ratcliff and Chandni Ravindan
Acknowledgements to the Wellcome Trust for funding

Background

During meiosis; the process responsible for production of haploid germ cells, the chromosome number is reduced. This is essential for the production of diploid zygotes upon fertilisation and thus forming a healthy embryo. Mistakes during the process of this reduction of chromosome number can lead to infertility, miscarriages and germline genetic disorders such as Klinefelter or Down's syndrome. Errors in the process become increasingly prevalent with female ageing with intense increases in aneuploidy (abnormal number of chromosomes) from 35 years. The reduction of chromosome number during cell division is mediated by a complex in which homologous chromosome pairs align, cross over and then separate. As an outcome, the synapsed homologous chromosomes form a meiotic 'bouquet' formation; whereby they adopt horseshoe-shaped structures through telomeric ends clustering on the nuclear envelope. This forms the structure by which chromosomes are held for segregation and progression through the cell cycle into metaphase.

The reorganisation of the homologous chromosome pairs is driven movement of the cytoskeleton, the movement is transmitted into the nucleus through the nuclear envelope by the LINC complex. During meiotic division, this LINC complex consists of SUN1 and KASH5 proteins. SUN1 associated with the telomeric end of the chromosome pair, spans the inner nuclear membrane, and via its C terminus SUN domain interacts with KASH5 KASH domain in the perinuclear space. KASH5 acts as a linker to connect the structure to the cytoskeleton by spanning the outer nuclear membrane- refer to figure 1. Disruption either protein in the LINC complex is a cause of infertility as chromosome synapsis is disrupted and thus accurate segregation of the chromosome pairs is inhibited.

No molecular information has yet been obtained for the meiotic LINC complex, and sequence comparison between the meiotic KASH5 and Mitotic KASH1/2 shows that there is little conservation between the SUN protein interacting KASH domains. Similarly SUN1KASH5 knockouts are infertile showing that the mitotic SUN/KASH proteins fail to rescue cells despite their expression in meiotic cells. This suggests that the SUN1-KASH5 molecular interaction is unique.

The aim of this project was to purify the SUN1 C terminal SUN domain both in a complex with KASH5 and not in the complex in order to investigate the interaction and structural properties of the SUN1 SUN domain.

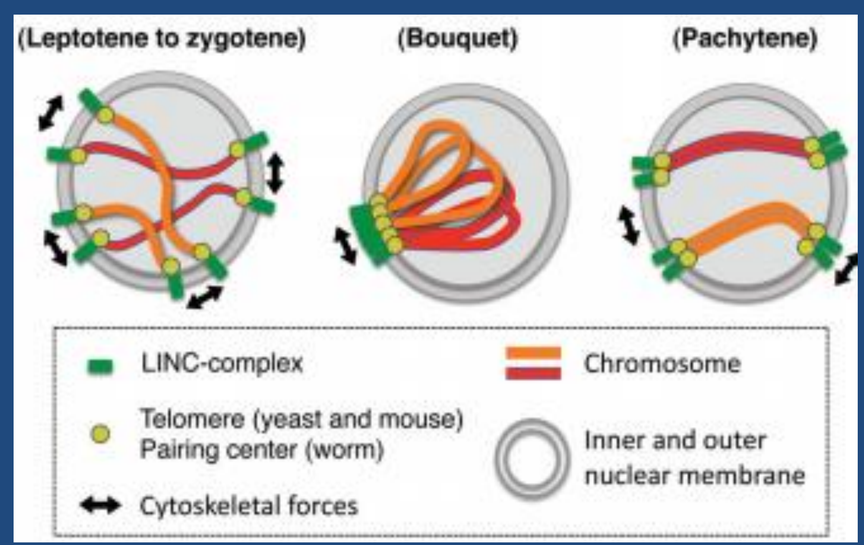


Figure 1- a schematic representation of the function of the LINC complex within a meiotic mammalian nucleus. The SUN1 complex is located on the inner nuclear membrane, and KASH5 spans the outer nuclear membrane where it is associated to the cytoskeleton.

Methods

- Bacterial cells were used as expression vectors containing plasmids with genes for SUN1 C terminal SUN domain with the aim to purify the protein. Multiple constructs of the protein were used, the shorter constructs (i.e. amino acids 616-812) forms only the SUN domain, where the longer constructs (i.e. amino acids 382/450/600-812) are likely to be involved in the formation of SUN1 multimers in vivo, however these longer constructs are less likely to be soluble in the grow up cultures. Solubility is necessary for the purification of the protein.
- Grow up co-transformations of SUN1 C terminal SUN domain and KASH5 C terminal KASH domain for purification. To allow the effect on solubility of SUN1 SUN domain to be compared both in a complex with KASH5 KASH domain and without.
- Since the SUN1 homology mitotic SUN2 is a trimer, a GCN4 protein which forms a trimeric coiled coil was fused to the SUN1 protein construct at the N terminus. This will increase the solubility of the SUN1 SUN domain which is necessary for purification.
- The SUN1 protein both in a complex and not in the complex was purified by nickel exchange and amylose exchange which was feasible due to His and MBP tags fused on the SUN1 and KASH5 construct respectively. After ion exchange was used to further purify the proteins which and the fractions containing the individual SUN1 or the SUN1 in a complex with KASH5 were pooled.
- With one sample of the complex and another of just the SUN1 complex a TEV cleavage was done. This removed the tags fused to the proteins for purification and also removed the GCN4 trimer forming region fused to the SUN1 construct.
- SEC MALS was then used to investigate the how much of the purified protein in the sample was a monomer or in a complex. This was done with both TEV cleaved and uncleaved proteins both SUN1 C terminus on its own and also SUN1 with KASH5.

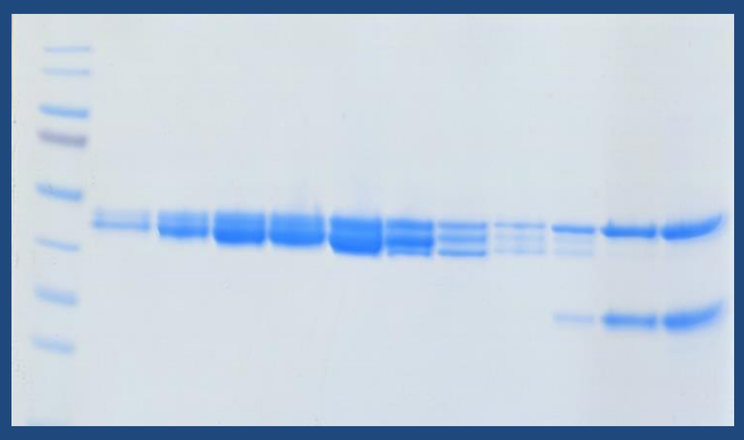


Figure 3- SDS PAGE of the ion exchange fraction 10-20 from a co-expression of GCN4 SUN1 616-812 and KASH5 542-562. The band further up the gel shows the KASH5 construct (with associated tags making it larger than the SUN1). When the KASH5 is not in a complex withSUN1 it is subject to degradation however stability increases dramatically when its in a complex. Elution bands 10-17 also show that KASH5 is able to exist as a soluble protein not in a complex with SUN1. Elution bands 19 and 20 suggest that SUN1 is only soluble when in a complex with KASH5 as it is not eluted on its own, only when in a complex. This could explain whySUN1 is meiosis specific.

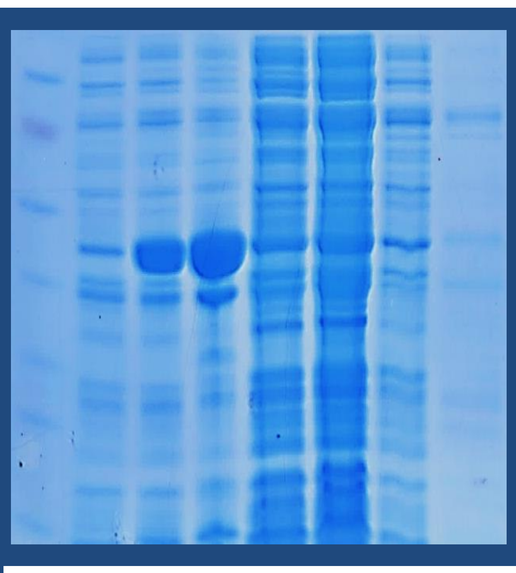


Figure 2- shows the SDS PAGE from the nickel purification of SUN1 450-812 which contains the SUN domain of the protein. From left to right the lanes are; protein ladder, uninduced sample, induced sample (using IGPT to stimulate the expression of the protein), pellet, supernatant, nickel column flow through, nickel column wash with 20mM imidazole, and nickel column elution with 200mM imidazole. Shoes that the majority of the protein is insoluble as it is seen in the pellet. Unfortunately the gel was not de-stained properly before imaging.

Results

- All of the cell grow ups with constructs composed of amino acids 382-812, 450-812, 600-812 and 616-812 of the SUN1 protein were all seen to be insoluble and therefore further purification could not take place. The protein constructs were insoluble due to cell stress and thus once expressed in the cells formed aggregates via interaction of exposed hydrophobic residues. Refer to figure 2 for an example of an SDS-PAGE of a SUN1 construct which was grown up and purified by a nickel column however was insoluble.
- SUN1 616-812 which is the N terminal SUN1 domain and therefore the protein construct contains no or little of the amino acid sequence which is involved in the multimeric formation which the protein adopts in vivo. GCN4 trimer forming region was fused to the N terminal region of the SUN1 616-812 construct. This promoted trimer formation- since the homologue, mitotic SUN2 is found in a trimer formation, this would therefore increase the solubility of the SUN1 construct allowing purification. This was seen to be mostly insoluble however there was a small amount of soluble material.
- Bacterial cultures were co-transformed GCN4 SUN1 616-812 and KASH5 KASH domain. This enabled us to see the effect on the solubility of the SUN domain in the presence of the KASH domain. A much higher proportion of the GCN4 SUN1 SUN domain was soluble when co-expressed with KASH5 KASH domain.
- During purification of the GCN4 SUN1 KASH5 complexes, it was apparent that the stability of KASH5 KASH domain is greatly enhanced when it is in a complex with the SUN1 SUN domain. This was shown by a high level of degradation of KASH5 in the ion exchange fractions which contained only the KASH5 which was not in a complex with the SUN1. This can be seen in figure 3. This ion exchange also suggested that SUN1 was insoluble when it was not in a complex with KASH5.
- SEC MALS was run on a few samples of purified protein from different grow ups. However the expression of the protein was very low so after the collection of the SEC MALS fractions, no further analysis could be done. With the GCN4 tag before it was cleaved off by TEV digest, the GCN4 SUN1 616-812 was found in a trimer and hexamer. In a ratio of 3:3 and 6:6 with KASH5. KASH5 was also shown to be a monomer on its own (figure 6) . GCN4 SUN1 construct was seen to be in a trimer- this is due to the GCN4 forming a trimer (figure 4) After TEV cleavage of SUN1 616-812 (therefore removing the GCN4 trimer forming region of the fused protein), SUN1 616-812 was seen to be a monomer which was expected as amino acids 616-812 have no/little multimeric forming region of the protein (figure 5).

Conclusions and Future Research

- Unfortunately much less data was collected from the placement as was expected, this was due to low protein expression and several issues with culture grow ups which held us back.
- The information obtained was only obtained once and therefore to confirm the findings, the experiments would need to be repeated.
- The SEC MALS data obtained from the cleaved and uncleaved GCN4 SUN1 616-812 suggested simply that the GCN4 caused a trimer formation and without the GCN4 fusion, SUN1 was a monomer. This suggested that the multimeric formation of the SUN1 may be different to that of its mitotic homologue SUN2 which forms a trimer. To understand the complex which the SUN1 protein forms in vivo, future research could involve the co-expression of longer SUN1 constructs with KASH5. This would promote the native multimer formation of the SUN1 whose solubility is seen to be increased by the presence of KASH5 KASH domain. This would allow for the complexes purification and structural analysis
- To understand further the ratio of SUN1 SUN domain and KASH5 KASH domain in the LINC complex in vivo, SEC MALS should be repeated to compare the ratio of the cleaved GCN4 SUN1 616-812 with the KASH5 as in our experiment using this sample, no protein was eluted.
- The crystal structure should be determined of the SUN1 C terminal SUN domain both in a complex with KASH5 KASH domain and without.
- This research could give way to the potential of microinjection in live cells which have faulty LINC complex and thus are unable to undergo meiosis. This could be done in vivo and promote the formation of successful germline cells which can then go on to be fertilised.

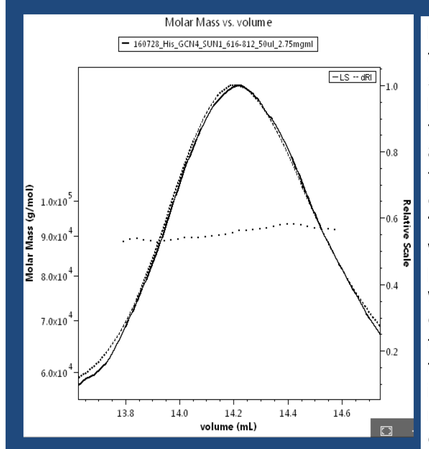
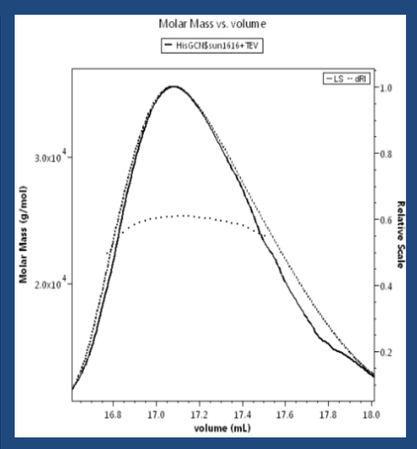


Figure 4 (above) shows the SEC MALS data obtained from the GCN4 HIS TEV SUN1 616-812. The peak size is 92.2kDa which corresponds to the protein construct in a trimer formation

Figure 5 (to the right) shows the MALS DATA for SUN1 616-812 (GCN4 fusion construct after TEV cleavage) which has a peak at 24kDa which corresponds to the Mr of the protein itself therefore not in a complex.



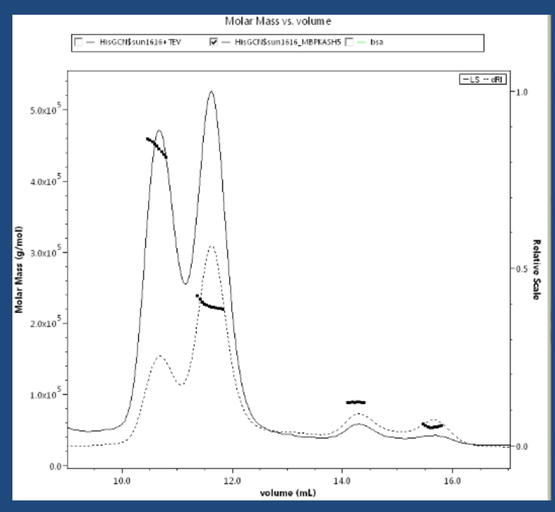


Figure 6 (to the right) shows the SEC MALS data from the GCN4 SUN1 616-812 co-expressed with KASH5 542-562. The first peak is 441kDa which corresponds to a 6:6 ratio of SUN1 and KASH5. The second peak is 222kDa which corresponds to a 3:3 ratio of the SUN1 KASH5 proteins in the complex. Peak 3 is 87.9kDa which corresponds to GCN4 SUN1 in a trimer conformation. The final peak at 53kDa which corresponds to a monomer of KASH5.

Supported by wellcome trust

References

Schmitt, J., et al., *Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope.* Proc Natl Acad Sci U S A, 2007. **104**(18): p. 7426-31.