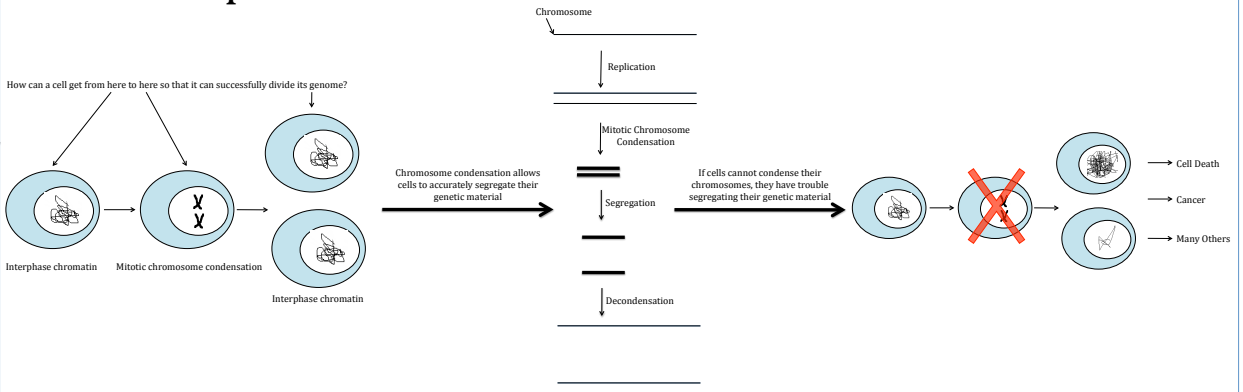
Searching for New Proteins Involved in Mitotic Chromosome Condensation

Andrew W. Grenfell, Amit C. Vas, Katie Furniss, & Duncan J. Clarke
University of Minnesota Department of Genetics, Cell Biology, & Development

Summary

The segregation of genetic material between newly formed daughter cells during cell division is a highly dynamic, intricately orchestrated cellular process. The sheer size of eukaryotic genomes poses a fundamental problem that must be overcome to ensure proper transmission of genetic material to daughter cells. After DNA replication, a typical human skin cell contains more than twelve feet of DNA, housed within a space measuring less than a millimeter across. Beyond simply containing this mass of DNA, cells must achieve a dramatic reorganization of their chromosomes during mitosis in order for it to be evenly divided between daughter cells. Mitotic chromosome condensation, the process by which chromosomes resolve into discrete bodies prior to nuclear division, must be faithfully carried out to ensure that each pair of sister-chromatids, the identical copies of each chromosome formed during DNA replication, disjoins during nuclear division. If chromosomes fail to condense during early mitosis, tangles between sister-chromatids can lead to breaks in the DNA or an outright failed segregation of sister-chromatids. Previous research has shown that topoisomerase II and the condensin complexes (condensin I and condensin II) are essential for mitotic chromosome segregation. However, a significant body of recent work suggests that there are more components to the chromosome condensation machinery than previously thought. In this work, I identify several novel proteins that could play a role in mitotic chromosome condensation. The identification of these proteins opens up new avenues of study, potentially providing the missing link between the physical phenomenon of mitotic chromosome condensation and our incomplete picture of the process on the protein level. Our understanding of mitotic chromosome condensation has been frustratingly vague to this point, with only a handful of proteins shown to actually play a part in this essential process. With the identification, and future verification, of these proteins as essential players in mitotic chromosome condensation, we will hopefully be poised to take further steps to understand this fundamental cellular process.

The Importance of Mitotic Chromosome Condensation

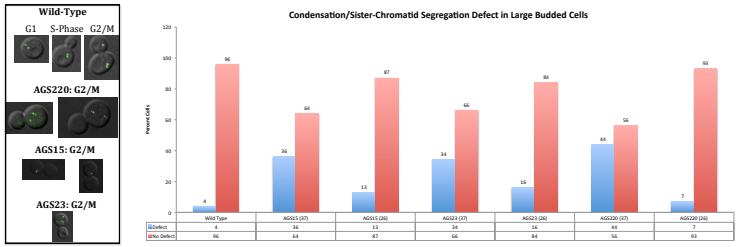


Conclusions

Possible Link Between Chromatin Remodeling and Mitotic Chromosome Condensation

- Each rescue plasmid contained at least one gene product with either intrinsic chromatin remodeling activity or multiple high probability interactions with chromatin remodeling factors
- The three major condensin targeting factors (AKAP95, TFIIIC, Scc2/Scc4) that have been identified have chromatin remodeling activity or are associated with chromatin remodeling factors
- Condensin was recently shown to interact with histones H2A and H2A.Z on chromosomes, this interaction is required for proper condensin targeting
- When paired with previous findings, the results from this study seem to suggest a link between local chromatin structure, usually associated with transcriptional regulation, and mitotic chromosome condensation.
- It is also possible that the gene products identified in this screen have function in mitotic chromosome condensation that is separate from their annotated function.

New Proteins Implicated in Condensation

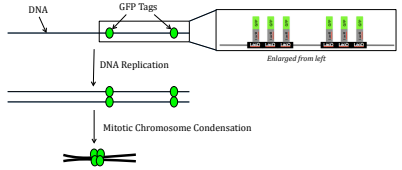


Strain	Plasmid	Gene	Function	Potentially Informative Interactions
AGS15	2-2	SPI1	GPI anchored protein involved in acid tolerance	
AGS15	2-2	UBP3	Role in chromatin silencing and ubiquitin regulation in conjunction with SIR4 and BRE5 respectively	BRE5, SIR4, NGG1, SPT4, HTZ1
AGS15	2-2	PET122	translational activator of COX3 in the mitochondrion	
AGS15	2-2	OXA1	Insertion of proteins into the inner mitochondrial membrane	
AGS15	2-2	BEM1	RHO-GAP with a role in controlling cytoskeletal dynamics and cellular morphology	SIT4, PCL1
AGS15	10-2	ELF1	zinc-finger transcription factor responsible for maintaining proper chromatin structure in transcribed regions	CDC73, LEO1, HIR1, SPT4
AGS15	10-2	APE2	Putative role in leucine extraction from dipeptides wthin the cell	
AGS15	10-2	RNC1	Plays a role in calcineurin regulation	
AGS220	37-4	GID7	Unknown function, linked to fructose-1,6-bisphosphatase proteasomal degradation	
AGS220	37-4	ATG22	Involved in amino acid efflux from vacuoles	
AGS220	37-4	SRO9	RNA binding protein with a possible role in actin regulation	BRE5
AGS220	37-4	GFD2	Unknown function, high copy surpressor of dph5 mutation	SAP155, SAP190, SIT4, TCO89, TOR1
AGS220	41-1	SEC15	Component of the functional exocyst complex which plays a role in exocytosis	
AGS220	41-1	SAP4	Required for Sit4p activity	
AGS220	41-1	ADE5/7	Required for purine synthesis	REB1
AGS220	41-1	TAN1	Predicted tRNA acetyltransferase	
AGS220	41-1	EMC4	Glutathione transferase activity	

Materials & Methods

A genetic screen using the LacO/LacR-GFP method of chromosome tagging was carried out using budding yeast as a genetically tractable model organism. Several steps allowed the identification of gene products with a potential role in mitotic chromosome condensation:

1. Random mutations were generated by UV mutagenesis and mutations causing temperature sensitivity were isolated.
2. Using the LacO/LacR-GFP system, the condensation state of the temperature sensitive mutants was assayed and phenotypes condensation defect strains were identified.



3. Genomic library complementation was used, and plasmids that rescued temperature sensitivity in the condensation defective mutant strains were isolated.
4. The above plasmids were sequenced, identifying genes whose products play a potential role in mitotic chromosome condensation

References: Belmont, 2006; Cherry *et al., 2012; D'Ambrosio et al., 2008; Earnshaw et al., 1985; Guacci et al., 1994; Hirano et al., 1997; Jensen et al., 2009; Paulson & Laemmli, 1977; Saitoh et al., 1994; Straight et al., 1997; Vas et al., 2007*

Acknowledgements: Many thanks to the members of the Clarke Lab, Duncan Clarke, Amit Vas, Katie Furniss, Andy Lane, Wei-Shan Hsu, and Hung-Ji Tsai, for their guidance throughout this project. Also, many thanks to the members of the Gardner and Luxton Labs, especially to Melissa and Gant for their help editing the text of this poster and of my Honors Thesis.