An evolutionary perspective of centromere composition

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Centromere Structures

- Centromere are important for providing structure and stability during important cellular processes including chromosome segregation.
- Without correct centromere composition cell viability decreases over time.
- However, related yeast species don't use a single conserved structure. Instead, there is a wide variety of centromere structures.
- Understanding how differences arise from species to species could lead to a better understanding of centromere composition and the tools involved in altering it.

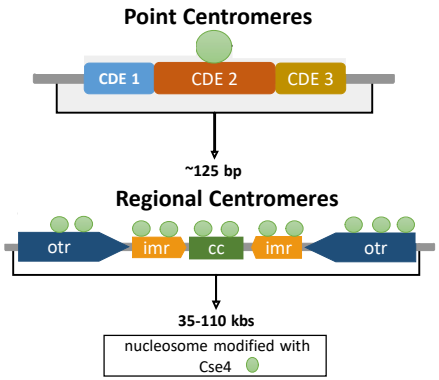


Figure 1. Centromeres contain specialized portions of DNA and special histone containing Cse4 (1)

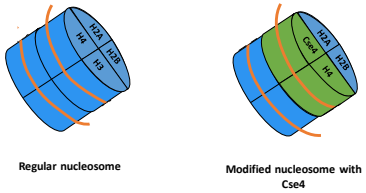


Figure 2. Cse4 histone modification (2)

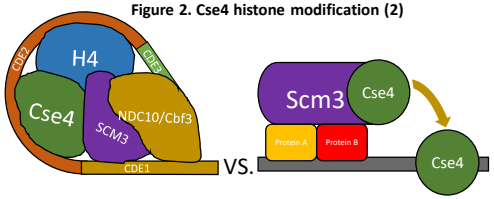


Figure 3. Point centromere Deposition vs Regional centromere Deposition. (3),(4)

- CENP-A/Cse4 is a histone variant that assists kinetochore development and faithful chromosome segregation.
- A histone Cse4-H4 dimer replaces the original H3-H4 dimer present.
- Correct localization of CENP-A/Cse4 in organisms from yeast to humans has been demonstrated as a requirement for chromosomal stability and long-term viability.

Cse4 and Scm3

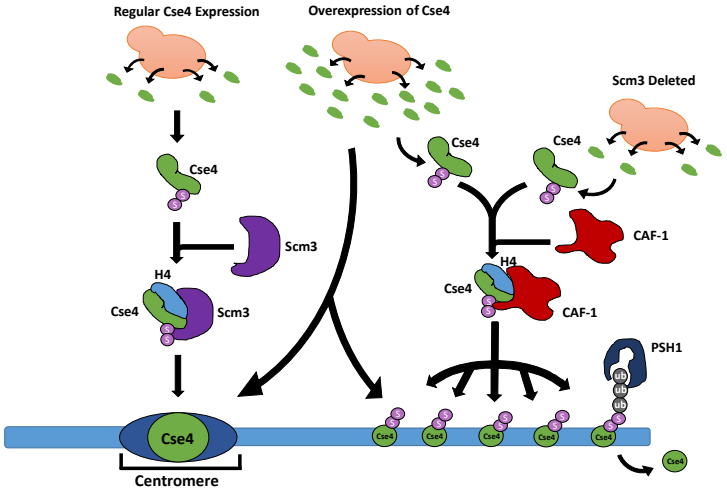
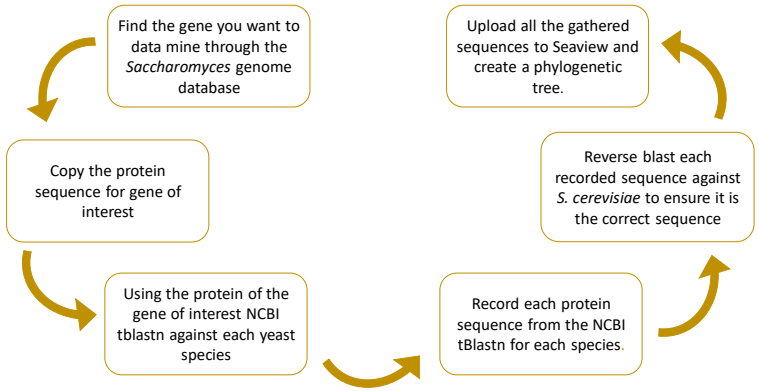


Figure 4. Cse4 SUMOylation an Scm3 chaperoning Shows two separate pathways for depositing Cse4 either through CAF-1 during Cse4 overexpression or Scm3 under normal conditions (2),(5)

- Our research sets out to understand how centromere function has changed overtime. Specifically, looking at the histone Cse4 and its chaperone Scm3.
- We want to understand how Scm3 is recruited to chaperone Cse4 and what circumstances lead to this recruitment as well as understand how these processes may change from species to species.
- Post- transcriptional modifications to Cse4 such as ubiquitination at sites K131/K155/K163/K172 and SUMOylation of K215/K216 Cse4 help regulate its proteolysis in non-centromeric regions to prevent mis-localization and maintain chromosomal stability(2)

Research Question
What tools have evolved to compensate for structural differences?

Methods



Evolutionary trends

- The results of our research show that overtime some yeast species have developed alternate ways of centromere maintenance and kinetochore development.
- A major question that has arisen from our research is Scm3 and its role in kinetochore development as well as how its recruited.
- Future steps would involve investigating Scm3 on a deeper level to better understand how its recruited inside the cell and looking into proteins that may replace known proteins responsible for kinetochore development in other yeast species.

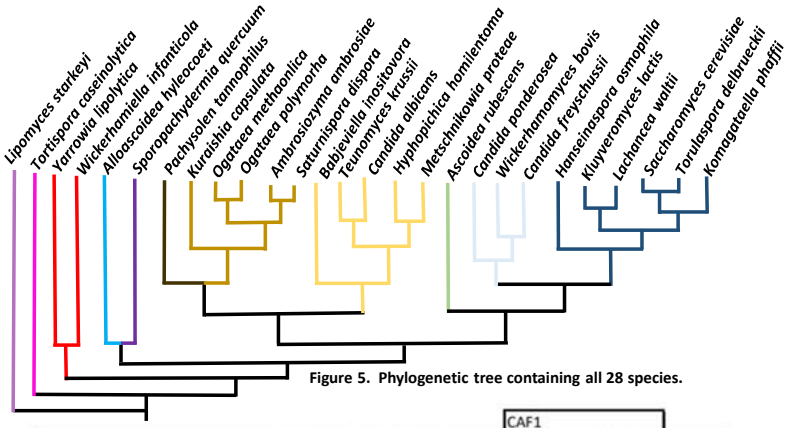


Figure 5. Phylogenetic tree containing all 28 species.

					CAF1			
Species	**Sir2**	**Cse4**	**Scm3**	**PSH1**	**RLF2p**	**MSI1p**	**CAC2**	**Ndc10**
Candida albicans	+++	++	+	+++	++	+	+	-
Kluyveromyces lactis	+	+	+	+	+	-	+	+
Lipomyces starkeyi	+	-	+	-	+	-	+	-
Ogataea polymorpha	+	+	+	+	+	+	+	-
Saccharomyces cerevisiae	+	+	+	+	++	+	+	+
Schizosaccharomyces pombe	+	+	+	-	+	-	+	-

Figure 6. NCBI blast results from 6 different species for genes of interest

Cse4 ubiquitination site

Yeast Species	K4	K131	K155	K163	K172
S. cerevisiae	K	K	K	K	K
A. rubescens	N/A	R	K	R	N/A
B. inositovora	N/A	R	K	R	N/A
C. albicans	N/A	R	K	R	N/A
T. delbrueckii	K	K	K	K	E
T. kruisii	N/A	K	K	K	N/A
S. dispora	N/A	K	K	R	N/A
M. protea	N/A	R	K	K	N/A
H. homilentoma	N/A	R	K	K	N/A
H. osmophila	N/A	R	K	K	G
S. pombe	N/A	R	K	R	N
S. dispora	N/A	K	K	R	N/A
W. bovis	N/A	R	K	R	N/A
W. infanticola	N/A	R	K	R	N/A
C. albicans	N/A	R	K	R	N/A
K. phaffii	N/A	R	K	R	N/A
K. lactis	N/A	R	R	K	T

Figure 7. Cse4 Ubiquitination sites in 17 Yeast species.

- After compiling all the protein sequences for Cse4 from every species we created alignments and looked for potential ubiquitination sites that may be shared from species to species
- What we found showed that K155 lysine is one of the most highly conserved across all the species.
- Differences in conserved ubiquitination sites point to variable ubiquitination pathways.
- How do variations in lysine sites inform our understanding of evolutionary divergence in yeast species over time?
- Do these variations drastically change ubiquitination activity and or protein function?
- Better understanding these differences could help us identify alternative areas to research and shed light on unknown protein interactions.

Acknowledgements

I would like to give a thank you to Dr. Jennifer Garcia and Dr. Sara Hanson For helping throughout the research as I attempted new methods and skills. I would also like to thank the Tutt library and their staff for their help in securing the resources I needed to complete the project from my home state of Arkansas. Funding was provided by the SCoRe program and the Molecular Biology Department. All the Datamining was done through the NCBI's tBLASTn program. All the alignments and trees were created using Seaview Software.

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