Role of KNL-1 in *C. elegans* kinetochore assembly

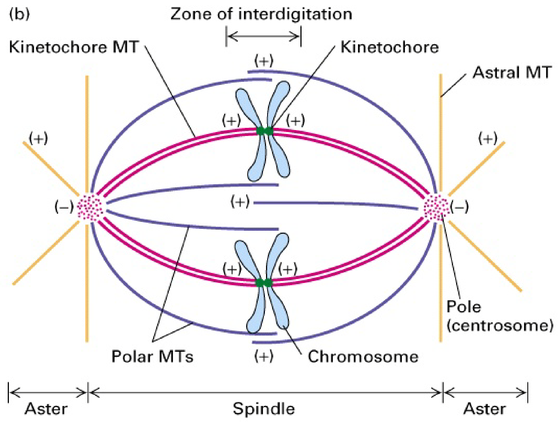
Nicolas Pogliano, Mark Moyle, Julian Espeut, Karen Oegema and Arshad Desai

Ludwig Institute for Cancer Research, University of California, San Diego

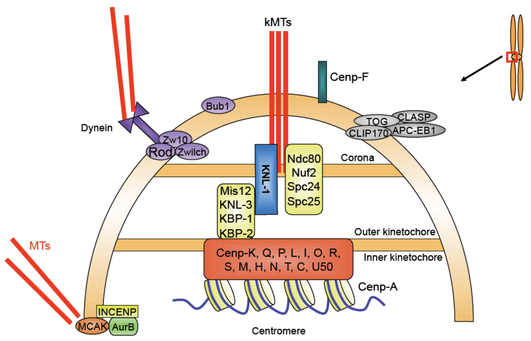
Abstract

The outer domain of the kinetochore builds an interface where dynamic microtubule interactions are integrated with regulatory pathways, such as the spindle checkpoint, to ensure high fidelity chromosome segregation. The conserved KMN (KNL-1/Mis12 complex/Ndc80 complex) network forms the core microtubule-binding site of the kinetochore and plays a central role in checkpoint signaling. In particular, KNL-1 is required to target multiple components important for microtubule-binding and checkpoint signaling. However, its mechanism of action and its interactions are not known. We used a yeast two-hybrid analysis to determine which domains of KNL-1 interact with GSP-1 and GSP-2 (Glc7-type phosphatase), homologs of the Protein Phosphatase 1 catalytic subunit (PP1c). PP1c localizes to kinetochores throughout the eukaryotic kingdom, where it is hypothesized to selectively stabilize correct kinetochore-microtubule attachments by counteracting Aurora B kinase on the adjacent inner-centromeric chromatin. We also tested for interactions with KBP-5 and Bub-1, a protein involved in the spindle assembly checkpoint.

The mitotic apparatus

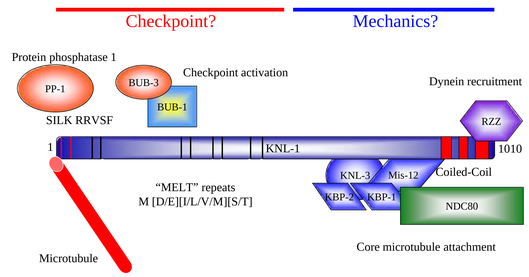


The kinetochore connects the DNA to the microtubules



KNL-1 plays a critical role in kinetochore assembly and function. It interacts with structural proteins and with checkpoint proteins that regulate mitosis,

What domain of KNL-1 interacts with GSP-1, GSP-2, KBP-5 and Bub1?



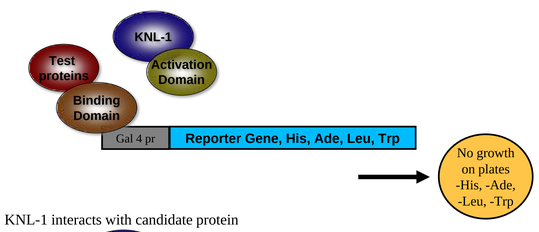
The Approach: Use the yeast two hybrid system to test the ability of KNL-1 domains and mutants to interact with candidate proteins

Do these proteins interact with:

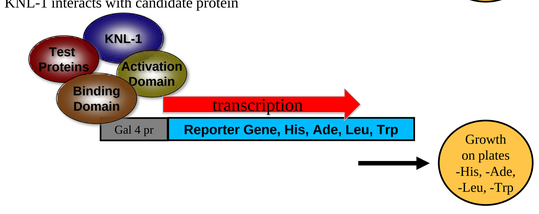
Bub1: Tyrosine kinase required for spindle assembly checkpoint
N-terminal and C-terminal domains tested
GSP-1: Serine/threonine specific protein phosphatase PP1, catalytic subunit
Full length protein tested
GSP-2: Serine/threonine specific protein phosphatase PP1, catalytic subunit
Full length protein tested
KBP-5: Localizes to kinetochore and interacts with KNL-1
Full length protein tested

The Yeast two hybrid system

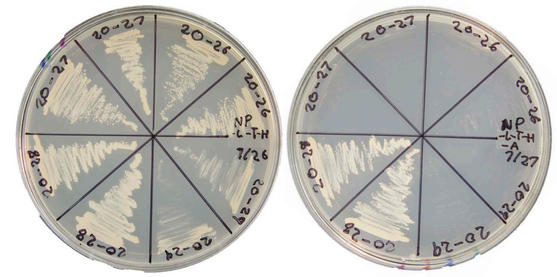
KNL-1 does not interact with candidate protein

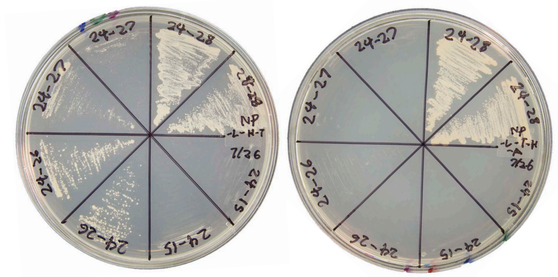


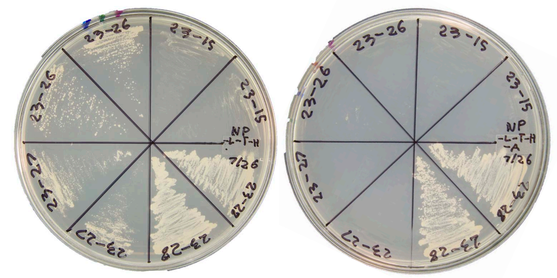
KNL-1 interacts with candidate protein



Yeast two hybrid results







	KNL-1 wt	KNL-1^{GANT}	KNL-1^{AILK}	Protein interacts best via:
GSP-1	+	+/-	+/-	GANT and SILK motifs
GSP-2	+	+/-	-	SILK motif
KBP-5	++	++	++	Unidentified motif, not SILK, GANT
Bub-1 NT	+/-	NA	NA	The C-terminal domain of Bub-1 interacts best with KNL-1
Bub-1 CT	+	NA	NA	

Conclusions

•It appears that GSP-1 and 2 interact with KNL-1 via the GANT and SILK motifs. Both motifs are required for interaction.

•KBP-5 clearly interacts with KNL-1, but does not require either motif tested. Additional mutations in KNL-1 should be constructed by site-directed mutagenesis to identify the amino acids in K NL-1 required for the interaction.

•The C-terminal domain of Bub-1 seems to interact best with KNL-1.