## Figures Accompanying: PUBLISHABLE SUMMARY 2<sup>nd</sup> term report Project No: 224874

**Project Acronym: Epicentromere** 

## Determining the Epigenetic Mechanism of Centromere Propagation

(4 figures total in this document)



**Figure 1. Cartoon of mitotic centromere organization.** Micrograph shows a cell in metaphase. The centromere is a unique locus specified by the histone H3 variant CENP-A (red foci) present on each chromosome (blue) that assembles the kinetochore that in turn directs attachment to microtubules (green fibres) during mitosis.

Cartoon shows a blow-up of the centromere complex with centromeric chromatin containing **CENP-A nucleosomes** that nucleates the constitutive **centromere complex** that in turn assembles the **kinetochore** during mitosis. The kinetochore is responsible for **microtubule** attachment that drives chromosome segregation during mitosis.



**to the cell cycle.** CENP-A containing nucleosomes (**red**) are interspersed with canonical H3containing nucleosomes (**green**) after replication in S phase. This mixed set of nucleosomes is the substrate for nucleating kinetochore assembly in mitosis and is maintained as cells exit in anaphase. **CENP-A** assembly initiates in telophase and proceeds through early G1 (presumably concurrent with removal of H3 nucleosomes). *Adapted from Jansen et al., JCB 2007* 





Figure 3. Cdk1/2 control timing of **CENP-A assembly.** A) Model illustrating Cdk1/Cdk2-mediated inhibition of CENP-A assembly, part exerted in through phosphorylation (P) of Mis18BP1 (member of the Mis18 complex) during S, G2, and M phases. Factors X and Y symbolize the involvement of other, yet to be identified, components. Inhibition is alleviated through APC/Cmediated loss of Cdk1 activity in anaphase, targeting the Mis18 complex to the centromere (licensing) followed by CENP-A assembly in G1 phase. Canonical (H3 containing) nucleosomes are shown in green, **CENP-A nucleosomes** in red. B) DT40 cells carrying an analog sensitive *cdk1* allele, either alone or in a *cdk2* knockout background are cell synchronized in G2 phase while a nascent CENP-A SNAP pool is labeled. Both lines assemble CENP-A at centromeres in G1 but only cdk1/2 double mutants have lost cell cycle control (with full blown assembly in G2 phase). Adapted from Silva et al. Developmental Cell 2012



**Figure 4. Rapid FACS based method to isolate gene targeted clones A)** Targeting design. **B)** Efficient isolation by FACS of CENP-A-YFP knockin clones. Image shows centromere localized YFP signal. (*Mata et al. PLoS ONE, 2012*). **B)** Fluorescence image of RPE cells carrying a CENP-A-YFP knock-in in a CENP-A knockout background. YFP fluorescence is found exclusively at the centromere where tagged CENP-A supports normal mitotic functions. **D)** Western blot of extracts form cells with indicated genotype. Cells in which one allele of CENP-A is deleted and one is tagged with YFP express only a high molecular weight CENP-A species and no endogenous CENP-A (**boxed in blue**). *Adapted from Mata et al., PLoS ONE, 2012*