Title of Project:	Using CRISPR Genetics, Synthetic Biology and Physical Modelling to understand mitotic chromatin compaction
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Summary of project
This project will use an interdisciplinary approach to characterise key differences in histone modification between interphase and mitotic cells that promote mitotic chromatin compaction. Previous work from our laboratories suggests that mitotic chromosome formation is driven by combined action of non-histone proteins complexes (e.g. condensin) coupled with changes in the spectrum of histone posttranslational modifications. The process involves not only dramatic reshaping of the chromatin to form recognisable chromosomes, but also a three to five-fold compaction of the chromatin. We hypothesize that this compaction is regulated by charge-shielding due to changes in histone post-translational modifications. The student will isolate histones from a range of wild-type and CRISPR mutant cells in G1, G2 and at various times during mitotic entry, characterise their evolving spectrum of modifications by mass spectrometry and assemble them into chromatin in vitro using either a highly regular model DNA template or on a more life-like native array with irregular nucleosome binding sites. Results of this biochemical analysis will be used to develop and refine predictive polymer models of chromatin folding currently under development. The model predictions will then be tested by monitoring the compaction of the chromatin by light and electron microscopy, biochemical methods (including sedimentation, nuclease digestion of the chromatin and a range of mass spectrometric analyses of intact and fragmented histones). When candidate modifications are identified, a synthetic biology approach will be employed using fusions with various archael enzymes (e.g. deacetylases, phosphatases, demethylases) to modify the chromatin modification status in vivo.

What quantitative skills will the student acquire or develop during their PhD project?
Culture and synchronisation of mitotic cells from wild type and a range of CRISPR mutants Isolation of additional CRISPR mutants and knock-ins as necessary Purification of histones bearing their native modifications in the Edinburgh Protein Production Facility Quantitative mass spectrometry to map histone post-translational modifications In vitro chromatin assembly on established and novel DNA templates being developed in the Gilbert lab Light and transmission electron microscopy to assess the conformations of the assembled chromatin Participation in molecular modelling of chromatin polymer structure and dynamics in the Gilbert lab Design and cloning of synthetic protein chimeras to artificially modify chromatin in vivo to test predictions of the models