Revealing a sequential order of events in relation to Spindle Assembly Checkpoint silencing through the development of "Mad2 pseudo-time"

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During mitosis, the Spindle Assembly Checkpoint (SAC) prevents chromosome missegregation by delaying anaphase onset in the presence of unattached kinetochores. Upon formation of kinetochore-microtubule attachments, the SAC is silenced on a per-kinetochore basis. However, how SAC silencing may be coordinated with other mechanochemical events in the kinetochore is not fully understood. To understand this further, we used fixed cell imaging in unperturbed pre-anaphase RPE1 cells, ordering individual kinetochores based on their levels of the SAC protein Mad2 to establish "Mad2 pseudo-time", used as a proxy for SAC silencing. We reveal that substantial Mad2 loss occurs early during Mad2 pseudo-time, likely a result of both dynein-mediated stripping and kinetochore dephosphorylation. Only after this loss does large-scale microtubule binding become apparent through mechanistic changes. Attachment maturation then occurs, with kinetochore-microtubule binding partners loaded and final silencing of residual SAC signal prior to anaphase initiation. This work provides new insight into the coordination of molecular events at the kinetochore, highlighting both graded and switch-like changes in kinetochore components over the course of SAC silencing.