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CHROMOSOMES

COMPLEX RELATIONSHIPS

Kinetochores assemble at centromeres to orchestrate chromosome segregation. ~100 proteins localize at centromeres and kinetochores, and although their assembly into complexes has been studied, a fully functional characterization is still missing. Earnshaw and colleagues monitored the entire proteome of chromosomes isolated from 11 chicken DT40 mutant cell lines depleted of defined kinetochore components. They used quantitative mass spectrometry to study the dependencies of 93 kinetochore proteins for kinetochore protein assembly and chromosomal association, revealing both predicted and unexpected relationships within the kinetochore network.

Depletion of various centromeric proteins (CENPs) led to the dissociation of kinetochore proteins in groups (cohorts), indicating that they form interdependent complexes. Depletion of CENP-C, which links centromeres to the outer kinetochore, severely disrupted outer kinetochore assembly. Moreover, NDC80 (a complex that connects kinetochores to the spindle) formed a central 'hub' for correlations between all groups of kinetochore proteins. Two of these cohorts had not been described before: one in the inner kinetochore, containing CENP-N, -L and -T; and one in the outer kinetochore, with the RZZ complex (ROD—ZW10—Zwilch) tightly associated with Spindly, CENP-E and MAD1. The latter may coordinate microtubule attachment with checkpoint signalling.

This study highlights the complexity of the kinetochore and reveals novel protein interactions that may prompt further biochemical and functional analyses. Importantly, it reports an approach to investigate complex inter-protein interactions in their natural environment without the use of antibodies or tags.

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ORIGINAL ARTICLE Samejima, I. *et al.* Whole-proteome genetic analysis of dependencies in assembly of a vertebrate kinetochore. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201508072 (2015)