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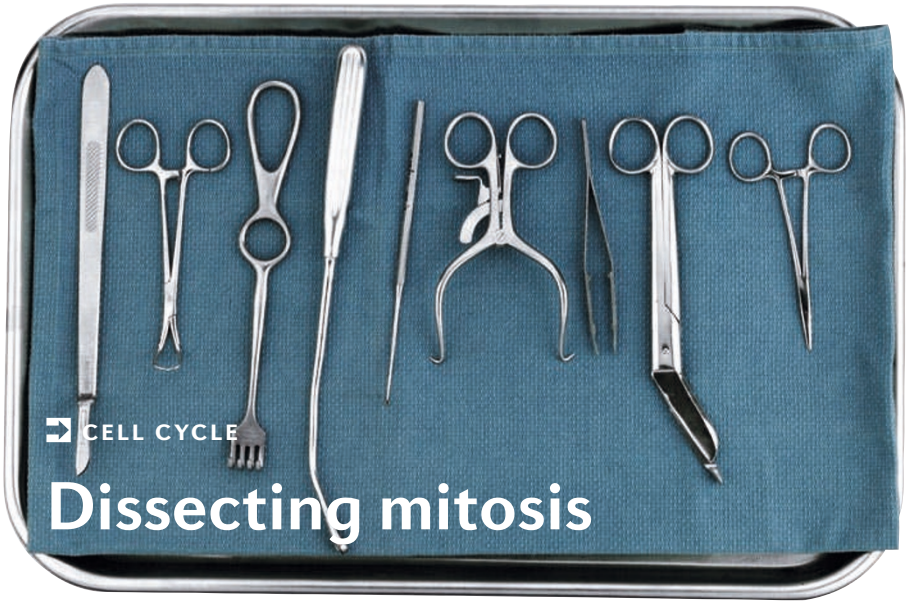

CELL CYCLE

Dissecting mitosis

Scientists from the MitoCheck consortium carried out high-throughput analyses aimed at assigning functions to all genes required for basic mitotic processes and assessing protein interactions. Using these approaches, they have identified previously undescribed genes and protein complexes involved in mitosis, as reported in *Nature* and *Science*, respectively.

Neumann *et al.* used small interfering RNAs (siRNAs) to target 21,000 genes in HeLa cells. The phenotype of the cells, which expressed histone 2B tagged with green fluorescent protein, was monitored by high throughput time-lapse microscopy. The ~ 190,000 time-lapse movies were analysed computationally by a program that recognizes changes in cell morphology. This allowed them to convert each movie into a 'phenotypic profile' that quantifies the effect of each siRNA. By scoring the cell phenotypes, the authors identified 1,249 candidate genes involved in mitosis. These were validated with two independent siRNA assays and narrowed down to 572 genes, less than half of which had previously been associated with mitosis.

To pinpoint the function of each gene, the authors examined the temporal kinetics of the phenotypes to associate them with different mitotic events. Based on this analysis and the severity of the phenotypes, they grouped the genes into two first-order phenotypic signature clusters, one for early mitotic defects and one for cytokinesis, which were further subdivided into clusters. They proposed that genes in the same cluster should have similar functions. Indeed, the cluster of genes with a phenotype indicative of defective spindle assembly contained genes known to be involved in this process. In addition, the cluster contained uncharacterized genes and genes with no known function in spindle assembly, such as torsin 1A-interacting protein 1 (*TOR1AIP1*), which encodes a nuclear membrane protein.

Data from these and other studies were used by Hutchins *et al.* to select candidate proteins that might physically interact during mitosis. They tagged 696 proteins with a combined localization–affinity purification tag in bacterial artificial chromosomes and stably expressed them in HeLa cells. The proteins were visualized using immunofluorescence imaging and separated into distribution patterns to identify potential protein complexes; the validity of the assay was confirmed by identifying known interacting proteins in the same cluster. Of the tagged proteins, 238 were purified and interaction partners were identified by mass spectrometry, resulting in the identification of ~ 100 protein complexes.

This approach identified three proteins, C13orf37, FAM128A and FAM128B (renamed MOZART1, MOZART2A and MOZART2B, respectively, by the authors), that interacted with subunits of γ-tubulin ring complex (γ-TURC; which is located at centrosomes and mediates spindle formation during mitosis) and might therefore be part of this complex. This was further suggested by the finding that the proteins localized close to centrosomes and on some occasions the mitotic spindle. Moreover, depletion of C13orf37 by siRNA interfered with normal spindle assembly; further analysis showed that C13orf37 is required for γ-TURC recruitment to centrosomes.

In addition to identifying genes and proteins involved in mitosis, the two studies have provided a wealth of publicly available information (accessible at the MitoCheck consortium website) that could be analysed to assess the involvement of the siRNA-targeted genes in other functions and to investigate other protein interactions.

Rachel David

ORIGINAL RESEARCH PAPERS Neumann, B. *et al.* Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* **464**, 721–727 (2010) | Hutchins, J. R. A. *et al.* Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science* 1 Apr 2010 (doi: 10.1125/science.1181348)