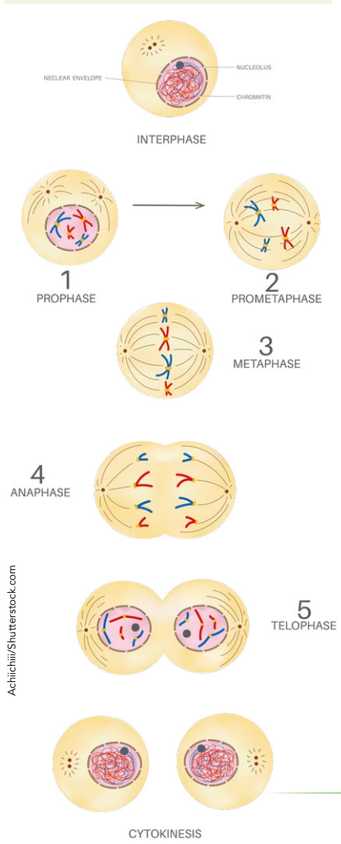
The intricate world of the centrosome

Dr Ryoko Kuriyama is a Professor at the University of Minnesota, Twin Cities. Alongside Dr Cody Fisher, she studies mammalian centrosomes, composite organelles responsible for the segregation of chromosomes during mammalian somatic cell division. Together, Kuriyama and Fisher, with the help of the United States National Science Foundation, investigate the detailed complexity of centrosome maturation, identifying the pericentriolar material protein Cep215 as a key player in the organisation of mitotic spindle poles during somatic mitosis.



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All cells must grow and divide to ensure their host's survival. In mammals, somatic cells proliferate via the cell cycle, which consists of two principal stages, interphase and mitosis (mitotic division, or the cell division phase). During interphase, cells produce proteins and organelles, and duplicate their genetic information. In mitotic division (mitosis and cytokinesis), duplicated DNA is segregated equally into newly formed nuclei and two new cells are produced. Mitosis itself comprises five stages: prophase, prometaphase, metaphase, anaphase and telophase. In prophase, two proteinous structures called 'centrosomes' migrate to opposite ends of the cell, and duplicated DNA strands start to condense. The classic 'X' shaped chromatids, so called 'sister chromatids' joined by a centromere – a small portion of DNA that binds the duplicated halves together, become apparent in prometaphase cells. Once the nuclear membrane that protects the genetic code in adult somatic cells dissolves, the cell next enters metaphase, and the condensed chromosomes line up in the middle of the cell.

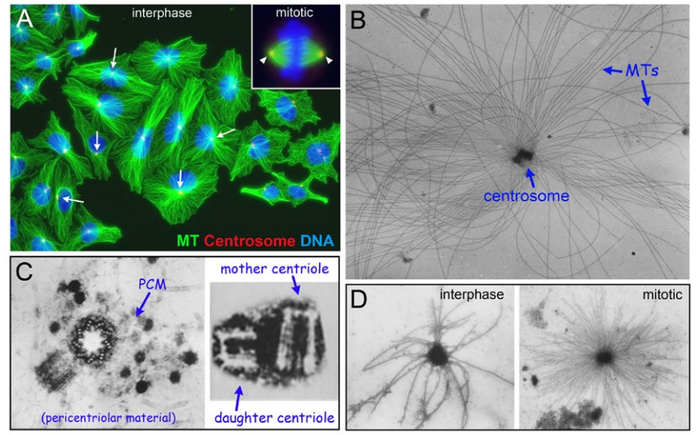
Microtubules – highly dynamic polymers consisting of stacked protein subunits (α/β-tubulin dimers) – are formed in dividing cells to make a machine called 'mitotic spindle' (A). Microtubules can be formed either inside the cell (A) or outside as membrane-bound cytoplasmic protrusions during interphase. If the latter, the structure called 'primary cilium' is formed from the centrosome. It serves as a sensory antenna by transporting molecules via protein motors, a class of molecular motors that utilise microtubules as highways. In spindles, some microtubules directly arise from the centrosome and extend to the chromosomes, where they attach to centromere-adjacent protein complexes called 'kinetochores'. During anaphase, the two halves of the chromosomes are pulled apart and dragged along the kinetochore fibres towards the centrosomes at the opposite poles of the cell. In the last mitotic stage, telophase, the split halves of the chromosomes unwind, and two new nuclear membranes form around each set of chromosomes localised at the cell poles. Finally, cytokinesis, or cytoplasmic division, follows mitosis to pinch the cellular membrane together down the centre of the cell, separating the single cell into two discrete entities, each with their own nuclear membrane, genetic information and one centrosome and spindle pole. Cellular division is complete, and the two new cells enter the cell cycle at interphase to begin the process anew.

THE CENTROSOME

The centrosome, a microtubule-organising centre (B), is one of the major players in mitosis. It is a complex organelle governing the intricate steps that dictate mitotic division. Each centrosome consists of a mother and daughter centriole, joined by an intercentriolar link (C). Centrioles are cylindrical structures composed of nine microtubule triplets. The centriole pair are surrounded by pericentriolar material (PCM), an electron-dense mass of varied protein complexes that regulate protein trafficking, degradation and nucleation (C). During the cell cycle, centrosomes, like chromosomes, are duplicated. On entering mitosis, these duplicated centrosomes undergo maturation, characterised by massive expansion of the PCM and the increased number of microtubules associated with it (D), whereby functional bipolar mitotic spindles are formed to pull apart the chromosomes. A plethora of compounds are involved in centrosome maturation, from protein mediators that facilitate molecular interactions, to motor proteins that dynamically build the spindle poles by transporting various molecules and structures.

DIPLOID DISORDERS

This tightly regulated molecular dance is essential for life, yet many of the mechanistic reactions involved in centrosome maturation remain elusive. Understanding the basic science of these interactions is essential, as aberrations in these processes can cause debilitating disorders, including cancers. Mistakes in centrosome maturation and subsequent chromosome segregation can also result in conditions such as microcephaly, a neurological disorder that hinders brain development, and primordial dwarfism, a genetic condition that impairs physical growth and intellectual development. Both disorders are lifelong and currently untreatable. Professor Ryoko Kuriyama and Dr Cody Fisher at the University of Minnesota have been investigating centrosome maturation, aiming to shed light on the roles and importance of proteins within the PCM. They believe that understanding the basic molecular mechanisms behind centrosome maturation will pave the way to medical intervention for aneuploidy-derived diseases, ultimately improving, and saving, lives.



A. Microtubules (MTs; green) in interphase and mitotic cells (inset) seen by fluorescence microscopy. Centrosomes (red) are located at the focal point of the interphase MT network (arrows) and mitotic spindle poles (arrowheads). DNA-containing nuclei and mitotic chromosomes are labelled blue.
B. The centrosome is a microtubule-organising centre. Electron microscopy shows *in vitro* polymerisation of microtubule onto the centrosome by incubation of isolated centrosomes with microtubule subunit proteins, tubulins.
C. Centrosomes are made of a pair of barrel-shaped centrioles and a surrounding fuzzy mass called 'pericentriolar material'. Mother and daughter centrioles are generally located perpendicularly to each other.
D. Centrosome maturation: mitotic centrosomes can polymerise more microtubules than interphase centrosomes.

CEP215: THE KEY PLAYER?

Of particular interest to Professor Kuriyama and collaborators is the PCM protein Cep215. Mutations in Cep215 are known to result in microcephaly, but how this protein functions during centrosome maturation, and why it is so essential to normal brain development, is still unclear. Homologs of the mammalian Cep215 protein appear widely across species, and such high-level genetic conservation indicates that this protein must play an important role in maintaining species' health and longevity. Cells lacking fully functioning Cep215 often display disconnected spindle poles and lack general microtubule organisation. Using a series of experiments, Kuriyama and her team set out to pinpoint the structure, function, and role of Cep215 within the mitotic cell cycle.

STRUCTURE SOLVING

To explore the structure of Cep215, the Kuriyama Lab examined the binding domains along the length of the protein. They cut Cep215 into a series of shorter peptides, including one they named 215N (E). By fluorescence labelling this peptide and visualising it within hamster cells, the team were able to confirm that 215N localises to the centrosome during mitosis. The strong fluorescence intensity of the 215N signal suggested to the researchers that 215N plays an essential role in targeting the whole protein, Cep215, to the mitotic centrosome.

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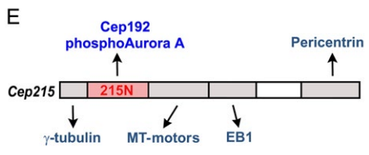
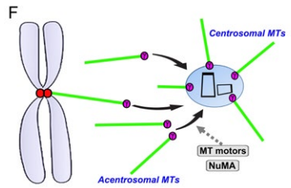
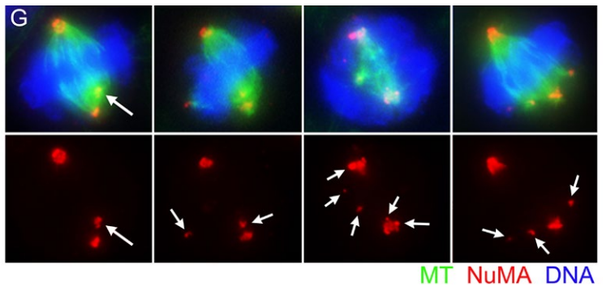
To help elucidate the function of the 215N domain at the centrosome, the team chose to explore its interactions with other molecules known to be involved in centrosome maturation. They previously reported close relationship between fly homolog of Cep215 and Aurora A (AurA), thus this protein kinase, required for spindle organisation during mitosis, was the first choice. AurA exists within the mitotic cell in both its active (phosphorylated, pAurA) and inactive (non-phosphorylated, AurA) forms. Using labelled antibodies against both forms of the kinase, they found that localisation of pAurA overlapped significantly with that of 215N at the centrioles, particularly around the proximal end of the mother centriole. The team hypothesised that, due to such significant overlap, these two molecules may interact at this location, and investigated this by depletion experiments. They used RNA interference (RNAi), a method in which short double stands of RNA are used to inhibit expression of the target gene, to first deplete mitotic pAurA, which resulted in undetectable levels of 215N, and then depleted 215N, which resulted again, in undetectable levels of pAurA. These results indicated that 215N and pAurA are mutually dependent upon each other for localisation to the centrosome during maturation.



E. Cep215 consists of different domains binding to specific molecules. A newly identified domain (215N) interacts with Cep192 and phosphoAurora A.



F. The centrosome is associated with two types of microtubules (MTs) at the spindle pole. Centrosomal MTs are directly polymerised onto the centrosome and acentrosomal MTs, formed independently of the centrosome, are transported to the centrosome by motor proteins and NuMA.



G. Cep215 depletion induces abnormal mitotic spindles (green) with dispersed NuMA (red; arrows).

PROTEIN CODEPENDENCY

After the interdependency of 215N and pAurA was confirmed, the team decided to explore whether 215N interacts with any other PCM proteins. They picked Cep192, a centrosome protein known to be involved in centriole duplication and microtubule nucleation onto the centrosome. By repeating their fluorescence localisation experiments, the team found that Cep192 and 215N co-localised identically at the centrosome. In repeating their RNAi depletion experiments, they found that in mitotic cells with depleted Cep192, 215N was undetectable at the centrosome. However, when 215N was depleted, Cep192 still localised well to the centrosome, indicating whilst 215N and pAurA are mutually dependent, only 215N is dependent upon Cep192. These three components are physically interacting with each other (E).

As the team had confirmed a relationship between the spindle-pole building protein Cep192 and the 215N peptide, they then investigated whether Cep215 played a similar role in forming spindle poles, by looking at the relationship between 215N and γ-tubulin, a microtubule-nucleating protein of the centrosomes. The researchers used RNAi to deplete 215N/Cep215 in mitotic cells and looked at the abundance of γ-tubulin, finding that up to 65% of γ-tubulin was retained in 215N depleted cells in comparison to control with 215N/Cep215. This is in striking contrast to Cep192-depleted cells where almost all γ-tubulin (~93%) disappears at the mitotic centrosome, indicating Cep215 clearly has only a minor role in γ-tubulin recruitment.

Studies like Prof Kuriyama's demonstrate the value and gravity of investigating basic biological mechanisms.

After showing that 215N interacted with proteins essential for microtubule formation during centrosome maturation, but played little part in polymerisation of centrosomal microtubules, the team sought to elucidate its true role in centrosome maturation. They did this by examining the phenotypes of mitotic cells depleted of the whole protein, Cep215. In cells without Cep215, they found a wide range of spindle pole abnormalities, from cells with short, thin, or singular spindles, to those with knots of disorganised spindles.

To examine these spindle knots, the team looked at the distribution of nuclear mitotic apparatus protein (NuMA), which plays a role in transporting microtubules assembled independently of the centrosome (acentrosomal microtubules), coalescing one of their ends into the pole and attaching spindle-pole fibres to the centrosome (F). Due to multiple unfocused spindle ends, where NuMA is present, disorganised spindle knots are likely to have highly randomised distribution of NuMA (arrows in G). This hypothesis was confirmed – the researchers noted that in cells depleted of Cep215 alone, as well as triple-depleted of Cep215, Cep192 and pAurA, an increased number of randomly distributed NuMA was observed. Kuriyama and her team suggested these results indicated that Cep215, Cep192 and pAurA act together as a protein complex in bringing the end of two types of MTs, centrosomal and acentrosomal, together by ensuring focused distribution of NuMA at the centrosome of each spindle pole (F), and therefore correct attachment of each pole to the centrosome.

Based upon these results, the authors proposed that the major function of Cep215 is to organise the functional spindle poles by connecting the mitotic centrosome with the spindle poles, via interaction of its domains, 215N being one of them, with other PCM proteins essential to centrosome maturation.

BASIC CELL RESEARCH: THE KEY TO DISEASE

This study serves to prove the complexity of centrosome maturation within mitotic cells. Centrosome maturation itself is just a small, but significant, process within one phase of mitosis, which itself is a stage of the wider cell cycle. Small changes in any molecules within this stage, and the cycle as a whole, can have huge impacts on human development, as exemplified by the disorders microcephaly and primordial dwarfism. If we don't understand these cellular processes deeply, we have no way of taking preventative actions against some of life's most debilitating disorders. Studies like Professor Kuriyama's demonstrate the value and gravity of investigating basic biological mechanisms.

Behind the Research

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Research Objectives

Research in Professor Kuriyama's laboratory centres on the cell cycle and cell growth control in mammalian cells. Using multidisciplinary experimental approaches, current efforts are orientated towards understanding the molecular mechanism and regulation of mitosis and cytoplasmic division.

Detail

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Bio

Ryoko Kuriyama is Professor emeritus at the University of Minnesota, Twin Cities. After completion of her Ph.D. in Biochemistry (University of Tokyo, Japan), she moved to the University of Wisconsin-Madison (Molecular Biology Laboratory) to study mammalian centrosomes before joining the University of Minnesota Medical School as a faculty member.

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Collaborators
Cody Fisher (University of Minnesota)

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Personal Response

Your research discovered a novel binding domain, 215N, on the Cep215 protein. Do you think this protein harbours further undiscovered domains that may help further elucidate its function?

"Yes, absolutely. Protein-protein interaction is a key to the mechanism of mitosis, which is a crucial process because the integrity of the genetic code is at stake. To execute this process seamlessly, spindle components must interact with each other via specific domains in a temporally- and spatially-dependent manner. Besides 215N, Cep215 contains domains capable of associating with molecular motors and pericentrin, a scaffolding protein in the PCM. These associations suggest that Cep215 recruits acentrosomal microtubules to centrosomes and that it helps to organise the expanding PCM structure. By identifying additional domains and their specific binding partners, we will have a better understanding of how Cep215 works in mitotic cells."