

Secrets of segregation

Cell biologist and yeast expert **Dr Peter De Wulf** gives the details of his work towards understanding the complex process of chromosome segregation and creating an intricate picture of kinetochore biology



Can you describe your professional background? What inspired you to research kinetochores and chromosome segregation?

After I obtained my MSc in Bioengineering and my PhD in Industrial Microbiology at the University of Gent in Belgium, I joined

Professor Lilia Alberghina's lab at the University of Milan to train in yeast cell biology. I then moved to the Massachusetts Institute of Technology (MIT) in the US, where Professor Peter Sorger was doing groundbreaking work on yeast kinetochores and chromosome segregation. I particularly loved his passion for bioengineering. For example, programmers in the lab wrote code for 4D microscopy to track fluorescently marked chromosomes in real time. He also liked to implement the most varied methodologies in his research. I spent five inspiring years at MIT studying yeast kinetochores. In my own lab, I similarly try to implement a multidisciplinary approach to answer complex questions in kinetochore biology.

You are looking at kinetochores from many perspectives. What approaches are you taking?

Human kinetochores contain up to 200 proteins assembled as in a perfect 3D puzzle. Because of its complexity, we study this structure in an experimentally more amenable species, the yeast *Saccharomyces*

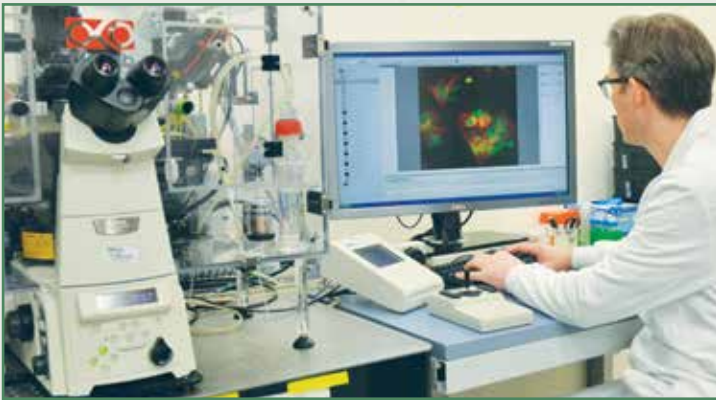
cerevisiae. We implement a broad spectrum of approaches to study how each protein contributes to the various activities of this megacomplex. For example, we use high-resolution live-cell fluorescence imaging of yeast lacking or overexpressing a kinetochore subunit to study its real-time consequences on sister chromatid-spindle binding and segregation. We also perform protein interaction screens and purify kinetochores to identify new subunits and regulators and to reveal how they interact with other proteins within the complex.

How do you confirm the data you gather from these approaches?

Out-of-cell reconstitutions of subkinetochore complexes, which are produced using bacteria, are used to confirm functional data. Reconstituted complexes are also valuable to study their activities under various conditions outside the yeast cell using biochemistry. Integrating out-of-cell data with cell biological data gives us a most complete picture of how kinetochore proteins mediate chromosome segregation.

Divide and conquer

Researchers at the **European Institute of Oncology** in Milan are looking at elements of the cell division process with a goal of ultimately identifying new targets for anti-cancer medicines



Spinning disk confocal fluorescence microscope (Perkin Elmer) that the De Wulf lab uses to image chromosome segregation in real time in dividing cells.

THE IMAGE OF a cell undergoing mitosis might be one of the most fundamental visual representations of biology at work, and one with which most people will be familiar. Yet this seemingly simple process becomes increasingly complex under examination. How do the duplicate chromosomes know to arrange themselves in just such a way to end up in separate daughter cells? How do they arrive in this position at the right time? And what force ensures that no chromosome is left behind? The process of cell division – and in particular the process of chromosome segregation – is fascinatingly complex and necessitates one of the largest and most complex molecular machines known to science: the kinetochore.

A dividing cell is controlled to a great degree by the spindle apparatus, a bipolar structure connected to each of the poles of the cell. From their polar centres, the mitotic spindles extend microtubules that radiate into the body of the cell. There they interdigitate, as microtubules attach head-on to sister chromatids, or laterally to each other. The microtubules that fix onto their opposing partners use motor proteins to exert pressure on each other, while the ones that attach to chromosomes do so via a kinetochore – a protein structure assembled on the centromere that acts as an interface between chromatid and microtubule. By shrinking and extending, the microtubules position the chromosomes in the middle of the cell, and it is only when this happens successfully that the kinetochore will give the signal for the final stage of cell division to begin. The spindles then pull the chromatids apart, moving away from each other and forming two new cells.

AVOIDING ANEUPLOIDY

Although there are many components involved in this process, perhaps the most fascinating is the kinetochore. It is not only a functional component in the segregation of chromosomes, but also a 'quality control' system for the process, refusing to initiate final cell division until the conditions are right. This assurance is vital to healthy cell division, but the kinetochores are not infallible. When chromosomes go astray, the result is aneuploidy – daughter cells with the wrong number of chromosomes. Aneuploid cells are usually not viable and quickly die – but if they do not, they can have terrible effects on the organism. Aneuploid cells can produce disorders

What has been the biggest challenge to date, and how have you overcome it? Has this opened up new research avenues?

We face challenges every day. They can be financial (limited research funding in Italy), scientific (hypotheses requiring reconsideration when not confirmed at the bench) or experimental (laborious experiments going wrong). Lying awake at night worrying about projects seems to be part of the job. For example, we had co-purified an uncharacterised, conserved protein with yeast kinetochores. Computational analyses indicated it was a regulator (an enzyme), not just another structural component. We studied it for six years, but in the end, it wasn't clear what it did at kinetochores. However, we noticed that this regulator also localised to another chromosome site, which was easier to study. In two years' time, we figured out what it did there, and it made sense that it would do the same at kinetochores. We went back to the kinetochore and our current data suggest that we are right. So, sometimes you hit a wall – but you have no choice. You must remain patient, stay positive, believe in yourself and never ever give up. In the end, you will succeed!

How important is collaboration to your research? With whom did you collaborate?

We definitely collaborate, as it is very difficult to master sophisticated techniques, and we don't necessarily have the very specific equipment to do a certain experiment. Foremost, we

collaborate with the technological facilities on campus. They are state-of-the-art and have expert faculty who help us with imaging and protein localisation analysis via microarray hybridisation, identification of co-purifying proteins and their phosphorylation state, etc.

From a basic science point of view, we collaborate on a daily basis with departmental colleague Dr Rosella Visintin who is a world-class yeast cell cycle expert. We organise lab meetings together and do a lot of brainstorming. We also collaborate with Professor Tony Hazbun at Purdue University in the US, who is an expert in protein-protein and genetic interaction screens. His work has helped us tremendously in understanding the functions of new kinetochore proteins. Professor Tomoyuki Tanaka at the Wellcome Trust Centre for Gene Regulation and Expression, University of Dundee, UK, is a world leader in yeast imaging. He has helped us with complicated microscopy experiments. He is a wonderful person, a scientist of the highest standard and an incredible source of ideas. I love discussing experiments, data and hypotheses with him.

INTELLIGENCE

CHROMOSOME SEGREGATION AND KINETOCHORE RESEARCH UNIT

OBJECTIVES

- To study chromosome segregation in order to understand how errors in the process cause chromosome number imbalance, a defect that underlies various syndromes and that characterises most solid tumours.
- To analyse the composition and regulation of protein complexes named kinetochores, and how they orchestrate chromosome segregation using baker's yeast as a model system.

KEY COLLABORATORS

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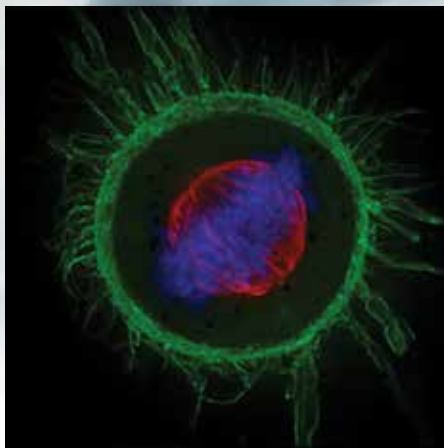
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DR DE WULF earned his MSc degree in Bioengineering and his PhD in Industrial Microbiology and Biocatalysis from the University of Ghent (Belgium). He was a post-doctoral researcher in signal transduction at Harvard Medical School (Dr Edmund Lin) and in kinetochore biology at MIT (Dr Peter Sorger). He currently holds a faculty position at the European Institute of Oncology (Milan, Italy).



Human metaphase cell with its replicated chromosomes (blue) aligned on the mitotic spindle (red). The cell membrane is colored green.

such as Down's syndrome, and in developed organisms, there is evidence to suggest that they are linked to cancer.

A lab in the European Institute of Oncology is using a variety of methods to study the nature and function of kinetochores in chromosome transmission. Led by Dr Peter De Wulf, the Milan-based lab uses yeast as an experimental model. As the chromosome segregation process is largely conserved between yeast and humans, yeast cells make the more attractive study subjects for a number of reasons. First, their cell division cycle takes only 90 minutes, whereas the human cycle occurs over a full day. Second, they are much easier to manipulate genetically and biochemically than human cells. After eight years of study, the team has uncovered many details of kinetochore function, all of which are just as applicable to human cells as they are to yeast.

MIXING METHODS

The lab implements a broad spectrum of methods to obtain a detailed understanding of kinetochore structure and function. To meet their goal of discovering new kinetochore proteins, De Wulf and his team isolate kinetochores from yeast and identify co-purifying proteins using mass spectrometry. The researchers then tag these proteins with green fluorescent protein and examine their intracellular localisation using high-resolution fluorescence microscopy to validate them as new kinetochore components. In order to discover whether a newly identified yeast kinetochore protein is conserved in humans, the lab computationally examines the human genome for sequence orthologues. Their kinetochore localisation is then verified in human cells.

In order to provide evidence of its contribution to chromosome segregation and the division cycle, the gene of the new yeast kinetochore protein is deleted or overexpressed and phenotypes (eg. chromosome-spindle attachment, chromosome movement along the spindle and arrest of the cell cycle process) are then assessed through live-cell time-lapse microscopy. These observations are complemented with a colony colour assay

that measures chromosome loss over several generations and thus quantifies a protein's contribution to chromosome transmission.

To determine where in the kinetochore a certain protein is located (known as kinetochore "mapping") and to link its position with activity, the scientists analyse its recruitment to centromeres by chromatin immunoprecipitation within a number of strains, each of which has had a single kinetochore subunit deleted.

In order to further associate function with structure, De Wulf's lab also overproduces a kinetochore protein or subcomplex in bacteria, purifies it and induces its crystallisation. X-ray diffraction analysis of the crystal structure can indicate how it interacts with other proteins, centromeres or microtubules, thereby supporting the mapping data. In addition, the protein might contain specific domains or folds that explain its function. Using its structure, the researchers can make pinpoint deletions or mutations in the protein to further phenotype it in yeast.

Another functionally comprehensive approach that underlines the strength of yeast as a model system involves performing genetic screens between yeast deleted in or overexpressing the given kinetochore protein and 5,000 other strains, each of which is deleted in a single protein. This gene-combination approach can reveal additive growth defects or growth rescue, indicating a functional relationship between the kinetochore protein and secondary genes.

KINETOCHORES AND CANCER

Integrating several approaches has proven very productive for De Wulf and his collaborators. They have discovered a number of conserved kinetochore proteins, including an essential kinetochore kinase and a protein complex that monitors excessive subunit concentrations and triggers their degradation to prevent disturbance to the kinetochore's structure and function. Understanding kinetochore homeostasis is very important, as many solid tumours contain abnormally high levels of kinetochore proteins. In fact, it has been shown that overexpressing just one kinetochore protein can cause chromosome missegregation and tumour formation in mice.

The strength of cancer cells is that they multiply aggressively and uncontrollably, thereby overtaking normal cells. However, if the division process is targeted, then their very strength becomes their Achilles' heel. In this respect, the proteins that constitute the kinetochore represent attractive targets for cancer therapies. Indeed, they are found nowhere else besides kinetochores and exist only in dividing cells. Targeting them would kill the aggressively dividing cancer cells with great efficacy. Because of improved specificity, targeting kinetochore proteins would reduce toxicity and side effects. In addition, the nearly 200 proteins of the kinetochore represent an almost endless supply of potential targets for this new therapy – which De Wulf is committed to accelerating. He explains: "In the long term, our studies aim to support the conversion of kinetochores into a next-generation cancer-drug target".