TIMELINE

Walther Flemming: pioneer of mitosis research

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The German anatomist Walther Flemming began his pioneering studies of mitosis almost 150 years ago. What were his achievements, and where have his discoveries led?

Browsing through the latest issues of cell and molecular biology journals, it is striking how many cover pages show images of dividing cells. This reflects the fact that research into cell division is at the forefront of the field. But what are the origins of this discipline?

It began in the seventeenth century, when Hooke[1], van Leeuwenhoek[2] and others discovered the *cellula* as a building block of many organisms. Then, in the first half of the nineteenth century, Schleiden[3] and Schwann[4] established the 'cell theory', according to which all organisms are composed of tiny units, the cells. Schleiden and Schwann assumed that cells are formed *de novo* from an intercellular substance in some kind of crystallization ('free cell formation') — an assumption that misled many scientists and inhibited research into cell division for almost three decades. For example, in 1875 Strasburger[5] published a comprehensive book *Ueber Zellbildung und Zelltheilung* ("About cell formation and cell division") in which he defended free cell formation. However, he had abandoned this idea by the time the third edition of his book was published in 1880.

By the 1870s, some scientists (such as Dumortier[6], von Mohl[7], Remak[8] and others) had shown that cells multiply by binary fission. At this time, Strasburger's colleague (and competitor) Walther Flemming (FIG. 1) was beginning detailed studies on dividing cells in different organs and organisms, mainly from the animal kingdom. Flemming's studies were not hampered by the idea of free cell formation, which he no longer believed in, and they eventually led to a solid foundation for modern cellular and molecular biology.

Flemming's career

Walther Flemming was born on 21 April 1843, in Sachsenberg/Mecklenburg in Germany. His father, Carl Friedrich, was a famous psychiatrist and neurologist. Flemming grew up in Sachsenberg as a shy but intelligent boy. Although his favourite topics were literature and philology, he decided to study medicine. He began his studies at the University of Göttingen and continued in Tübingen, Berlin and Rostock. During his training in the clinic at Rostock, Flemming studied histological and zoological preparations under the guidance of Franz Eilhard Schulze (who was himself strongly influenced by his mentor, Max Schultze, one of the first 'cell biologists'). From Schulze, Flemming learned constructive criticism, the cautious evaluation of results and the avoidance of speculation — all of which were characteristic of his later scientific work. Other features of his research included careful observation, frequent controls and a thorough evaluation of all results. Flemming was also influenced by Rudolf Virchow, one of his academic teachers, and Max Schultze's students Wilhelm Kühne and Gustav Schwalbe, who implanted in him the idea of the cell as the fundamental, autonomous unit of life.

Figure 1 | **Portrait of Walther Flemming.** A well-documented appreciation of Flemming's work is given in *The Birth of the Cell* by Henry Harris[36]. (Image provided by the Science Photo Library.)

For short periods Flemming assisted in anatomy and histology in Würzburg and Amsterdam until, in 1870, he was offered the position of *Prosektor* (leader of dissections and anatomical preparations) in Rostock. He also taught histology and comparative anatomy, and his students were enthusiastic about his talent for drawing, which brought cells, organs or organisms to life on the blackboard. Indeed, all of his later publications were illustrated by fine detailed drawings that aided understanding (FIG. 2). At the end of 1870 he presented his *Habilitation* thesis about connective substances and the vessel wall in molluscs, to become *Privatdozent* (academic teacher).

In February 1872 the head of anatomy at Rostock, Wilhelm Henke, asked Flemming to go with him to the German University of Prague, where Flemming was responsible for all histological lectures, seminars and courses. Here, in the same institute as Johannes Evangelista Purkinje, who was considered the father of histology, Flemming began his detailed investigations into cell division. Since the German revolution of 1848, nationalism had been growing all over Europe, and Czech students passionately demanded a Czech University in Prague. So the climate became increasingly hostile until most German professors preferred to return to Germany. Although Flemming was not called to the Chair at Königsberg (East

Box 1 | Cytoplasm and mitochondria

One of Flemming's favourite topics was the structure and function of the protoplasm. During his careful observations, particularly in the 1880s (REF. 37), he used optimal fixations and different staining procedures to show that the protoplasm has a mainly filamentous appearance; this contradicted the widely accepted proposal by Carl Frommann and Karl Heitzmann of a granular and reticular substructure. Flemming defended his *Filartheorie* ("theory of a filamentous structure") vigorously, and surrendered only when he was too ill and weak. In 1898, however, Carl Benda used a special fixation and staining method to show elongated corpuscles in the protoplasm. He termed these mitochondria because of their tendency to form chains. Flemming's assistant Friedrich Meves[38] later showed, shortly after Flemming's death, that Flemming was not completely wrong — Meves identified Flemming's 'filaments' and Benda's mitochondria as one and the same.

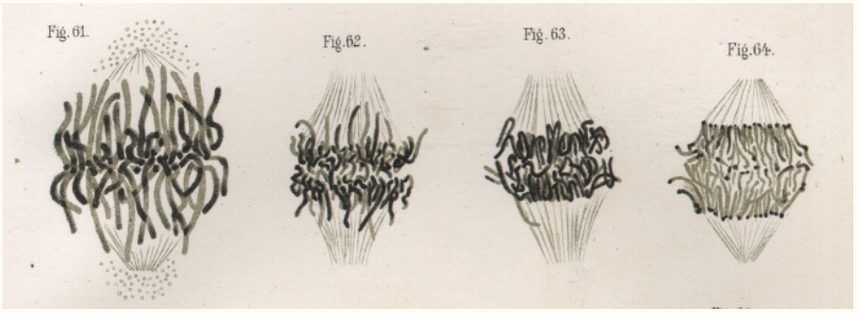


Figure 2 | **Illustration from *Zellsubstanz, Kern und Zelltheilung*[22].**

Prussia), as he had hoped, he was recruited to the vacant Chair of Anatomy at Kiel (Schleswig-Holstein). Almost all the medical faculty voted for Flemming. However, during negotiations, one faculty member strongly recommended Friedrich Merkel, an anatomist from Rostock, who was the son-in-law of a well-known German anatomist. Nevertheless, Flemming took up the position in February 1876.

Although the Christiana Albertina University in Kiel was very small, the old institute of anatomy was not big enough for the 70 or so medical students. There was not enough money to buy new microscopes and other equipment, and, at the beginning, Flemming took charge of all lectures, seminars and courses without assistance. He had to do battle with the university's administration; these struggles for resources were a heavy burden for Flemming, a peace-loving man whose students loved him for his cordiality and benevolence. In his late forties, Flemming developed a severe neurological disease from which he did not recover. At the turn of the century, his illness became so severe that he had to retire and, on 4 August 1905, he died aged 62 in Kiel[9]. By this time, however, Flemming's institute had become a leading centre for research into histology, cytology, comparative anatomy and, in particular, mitosis.

Initial studies

When Flemming began his research, cell biology was just beginning to boom (TIMELINE). In 1833, even before Schleiden and Schwann had presented their cell theory[3,4], Robert Brown[10] had described an ovoid in the cell as the "nucleus", and Dumortier[6] and von Mohl[7] had discovered binary fission of the nucleus and cell. Remak[8] gave the first descriptions of the changes that occur in the nucleus, and Purkinje[11] underlined its importance and the requirement for this organelle throughout the life of a cell. But in 1868, at the beginning of his career, Flemming — whose knowledge of histology was derived mainly from zoological objects — was interested in the sensory organs of molluscs. He also studied adipose tissue, and clearly stated its character as connective tissue; before this, adipose tissue had been considered to be a separate organ. Flemming also analysed lipid droplets as products of cellular metabolism. In addition, he was interested in the involution of adipose tissue, and studied the fine structure of the fibres of connective tissue and their swelling during treatment with acids.

At a time when the focus of Flemming's interest was still the behaviour of individual cells, research into the process of cell division had already begun. In 1873, Schneider[12] sketched the important steps of cell division. He saw the transformation of the nucleus into rod-like structures (*Stäbchen*), which assembled in the centre of the cell (at what we now know as the metaphase plate). At a stage that we now call anaphase, two groups of *Stäbchen* could be seen in the elongated cell.

Between 1874 and 1876, Flemming described these steps in more detail[13–15]. Whereas Schneider[12] had postulated that the nucleus undergoes deformation during cell

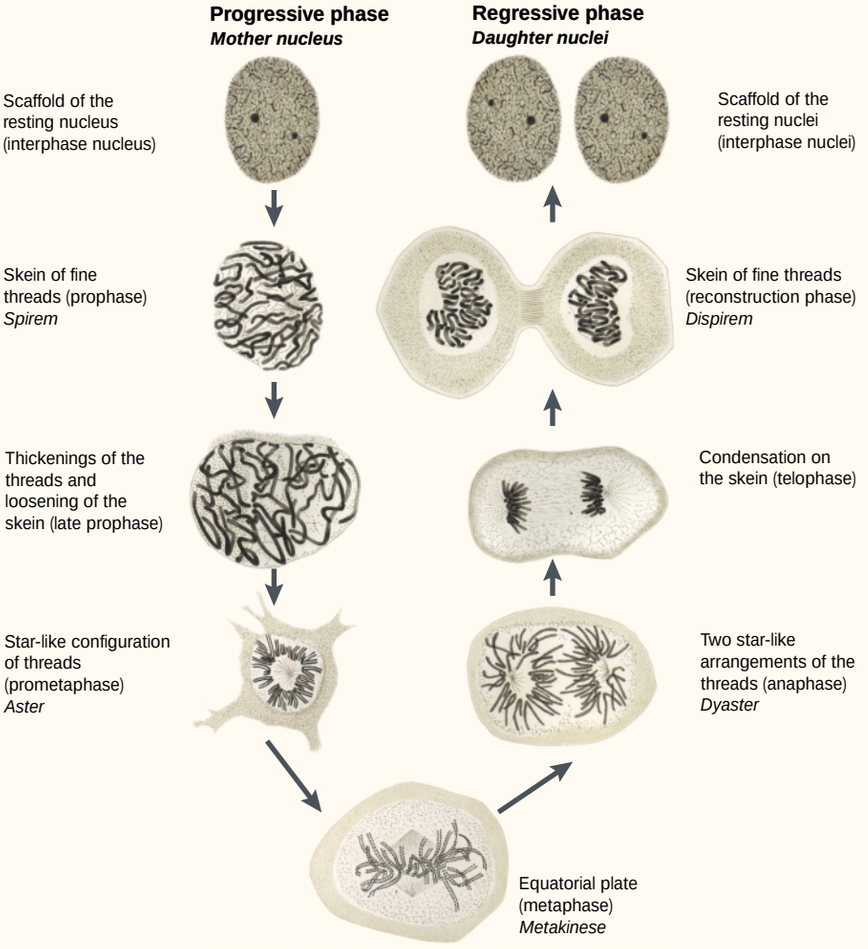
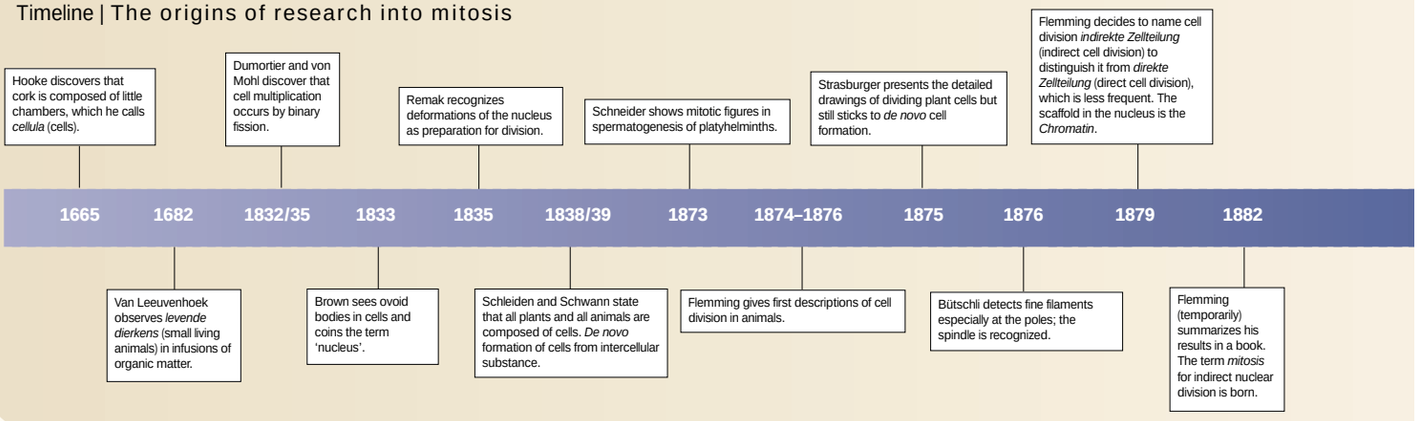


Figure 3 | **The progressive and regressive phases of cell division.** Mitosis starts with the skein-like form of the nuclear threads (prophase), which changes into the aster (star-like configuration of the threads at prometaphase). This stage moves into the equatorial plate (metaphase), which then immediately forms the double star (anaphase). When the threads have reached the position of the daughter-cell nucleus, the double skein (telophase) can be observed. (Images reproduced from REF. 22).

Timeline | The origins of research into mitosis



multiplication, Flemming showed that the scaffold and network within the nucleus transformed into 'threads', which then separated into two groups. These two groups, in turn, formed two skeins, from which the scaffold of the nuclei reappeared. By carefully studying wounds and scars, Flemming and his students found an accumulation of dividing cells in these tissues, and concluded that the regeneration of tissues and organs occurs by cell division[16].

At that time, no general repertoire of histological methods existed — indeed, one of the first monographs on histological methods, by Alfred Fischer[17], was not published until the end of the nineteenth century. In this book, many studies of fixed cells were considered to be based on artefacts, so Flemming had to spend a long time designing methods to facilitate his observations[18,19]. He experimented with various acids to find an appropriate fixative for preserving the fine structure that he had seen in the living cells and finally used a mixture of chromic, osmic and glacial acetic acids, which was soon adopted by colleagues and known as 'Flemming's solution'. He tested haematein and haematoxylin for their usefulness as dyes, and also found that the addition of very low concentrations of picric, acetic or formic acid to the medium best brought out the structures of the nuclear scaffold and the fine structure of the protoplasm (cytoplasm; BOX 1).

Nuclear division

In 1878 and 1879, Flemming published two important papers[20,21], in the second of which he coined the term 'indirect nuclear division' because he had observed that a transformation of the nuclear content had to take place before fission could occur. (A cleavage of the nucleus and protoplasm — which, until then, had been generally assumed — was called 'direct nuclear division'.) The methods that Flemming had developed allowed him to recognize a fibrous scaffold in the nucleus, which could easily be stained and was therefore named *Chromatin* ('stainable material'). Some other structures remained unstained and were therefore termed *Achromatin*. These results led, in 1882, to the publication of Flemming's comprehensive book *Zellsubstanz, Kern und Zelltheilung* ('Cell substance, nucleus and cell division')[22], which became the foundation for all further research into mitosis. Although Schleicher[23] had proposed the name *Karyokinesis* for this process, Flemming decided to use a more exact term, and he called the observed alterations within the nucleus *Karyomitosis* (meaning threadlike metamorphosis of the nucleus). He christened the arrangements of the nuclear threads *Mitosen*. Only afterwards, in 1888, did Heinrich Wilhelm Waldeyer[24] coin the term *Chromosomen* ('chromosomes', meaning stainable bodies) for Flemming's nuclear threads.

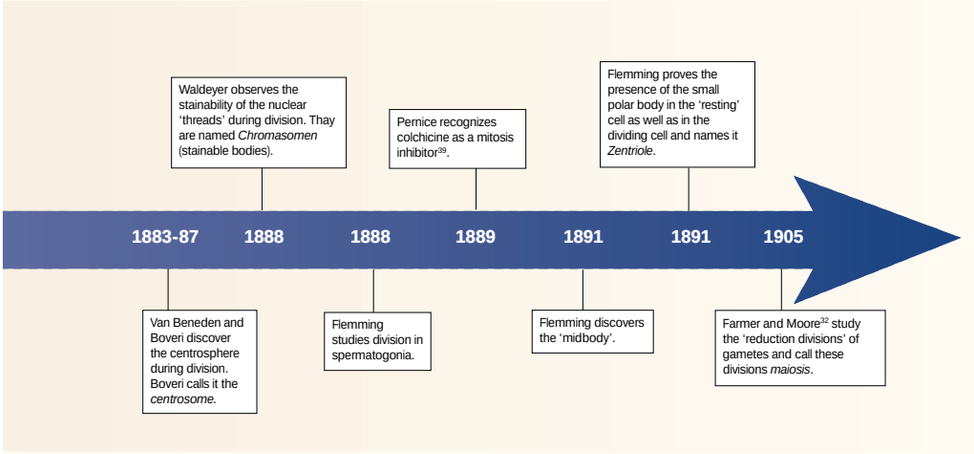
Flemming described the processes in the nucleus as we know them today, and he made a distinction between the 'progressive' and 'regressive' phases of cell division (FIG. 3). The progressive phase started with the appearance of the threads in the nucleus of the mother cell and continued as far as the arrangement of the threads in the centre of the cell. The regressive phase, by contrast, began with the separation of the threads into two groups and ended with the reappearance of the daughter nuclei.

Although Flemming had the correct idea that the chromatin network in the 'resting' nucleus transforms into the threads (chromosomes) — thereby representing continuity of the nuclear material — he did not have the techniques or equipment to prove this. The objective lenses of his microscope were composed of lenses with different refractive indices, but these lenses contained many aberrations — in particular, the chromatic aberration often delivered structures with coloured halos. Moreover, the illumination was not yet very bright and depended strongly on the intensity of the daylight. The microscopes had no sophisticated condenser systems, so it was not possible to produce a pseudo-phase-contrast image. But Flemming's drawings clearly showed correct images of the spindle apparatus, for example.

In 1891, Flemming published a paper[25] describing the remnants of the spindle just before complete cleavage. He called this the *Mittelkörper* or midbody and considered it to be an equivalent of the cell plate in plant cells. Otto Bütschli had shown earlier[26] that a fibrillar structure becomes visible, which he called the pole aster. Edouard van Beneden[27] and, almost simultaneously but independently, Theodor Boveri[28] had found a tiny structure at the pole, which they both termed the *Polkörperchen* (polar body), but they had assumed that this formed *de novo* during cell division. Also in 1891, in a sensational paper[29], Flemming showed unequivocally that this body is not formed anew but persists, and he coined the term *Zentralkörperchen* (central body) or *Zentriol* (centriole). He was convinced that the filamentous structure of the spindle in mitosis was responsible for transport of the threads, but again he could not prove this. His delicate observations on the behaviour of spindle fibres were later confirmed by electron microscopy.

Division during development

In his attempts to present a general interpretation of mitosis that was valid for all organisms, Flemming also studied division during the development of spermatozoa; he described this in a lecture in 1888 (REF. 30). Although Flemming failed to recognize the



differences between the division of somatic cells and that of gametes, as he reported in his paper of 1882 (REF. 31) he had already observed the paired nature of the chromosomes in the early stages of spermatozoan development. In 1905, Farmer and Moore[32] reported the first descriptions of *maiosis*. Strasburger[8] assumed that the rod-like structures (chromosomes) were transversely split, and this was a source of strong controversy between him and Flemming. Flemming insisted — and could prove — that, in *Metakinese* or earlier, the threads were split longitudinally. He had already assumed[31,33,34] that one half of this longitudinally split pair was destined for one daughter cell, whereas the second half went to the other daughter — a prediction that has turned out to be correct.

Consequences of Flemming's findings

A host of papers appeared over the two or three decades after Flemming published his spectacular book on mitosis[22]. But research into mitosis then slowed down until around the 1920s, once Alfred Fischer's book[17] had warned about the danger of studying artefacts caused by fixation and staining. For example, for some time the spindle fibres had been considered to be coagulation artefacts produced by fixation. In the mid-1920s, Karl Belar experimented with dividing spermatocytes to find out the mechanics of chromosome transport and, in 1929, he proposed the stem body hypothesis[35]. A few years before the Second World War, a new age of mitosis research began. This was interrupted by the war — especially by the holocaust and the emigration of many Jewish scientists from Germany.

Flemming could not have foreseen the variety of disciplines that have come out of his work. First, of course, are the fields that are closely connected with the original mitosis research. Chromosome structure and function has become a special branch of this, leading to investigations of kinetochores and telomeres for example, and even to the discovery of the function of the nucleolus. The combination of mitosis research with breeding experiments to explain Mendelian inheritance finally resulted in genetics and cytogenetics, which, in turn, led to gene manipulation, gene therapy, mutation research and the deciphering of the genetic code.

The spindle is still a structure of interest. Research is being done into its behaviour during division, into its function as an apparatus for transporting chromosomes, microtubules, tubulin, microtubule-associated proteins and motor proteins, and into ciliary movements, the centrosome (centriole) and mitotic poisons (used as cytostatic agents). Other fields include the 'uncontrolled' growth of cancer, and cell-cycle regulation. Last, Flemming's research has also led indirectly to studies into programmed cell death, which starts with drastic changes in nuclear structure and cell-cycle regulation.

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