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a chromosome's glue

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We all begin with one cell, which divides into two – and so on. It sounds straightforward but a cell has various components (nucleus, mitochondria, Golgi apparatus…) each of which carries out vital activities. If two daughter cells are to survive, they must receive a copy of each component from the mother cell. A mother cell cannot just split in two, pour half of its contents into one cell and tilt the rest in the second. That would be like producing two cars of the same make where one is built with no engine and the other with no wheels. Every part of a cell has a specific and an essential role, which is why each part has to be inherited by progeny. Among these essential components daughter cells must receive a copy of their mother's DNA. The only way to do this is for the mother cell to double its DNA and then distribute it in such a way that the DNA in each daughter cell is identical in quantity and nature. This can occur thanks to a mechanism known as mitosis. During mitosis, a dividing cell's chromosomes (its DNA) alternate between two opposing states: individualized and clustered. It turns out that a protein – already known to scientists – is directly involved in the making of these two chromosomal states. Its name? Ki-67.

"Chromosomes Chromatids" by Jenny Gray

Courtesy of the artist

Mitosis is the process by which cells divide while distributing their contents – in particular their DNA – in a balanced manner to the two daughter cells. Mitosis is billions of years old since the first form of eukaryotic life used it. As a consequence, the process has had plenty of time for refinement, and the intricacies and beauty of its various stages is awe-inspiring. Names have been given to each stage: prophase, metaphase, anaphase and telophase – with an in-between stage called interphase when not much happens. Without going into any detail whatsoever, when a cell is not dividing (interphase), its chromosomes are kept (and protected) within an organelle known as the nucleus. When a cell is dividing (prophase to telophase), its nuclear envelope disassembles thus freeing the chromosomes (that have just been doubled), which are then dispatched to the daughter cells who rapidly reform their own nucleus to protect their own batch of chromosomes.

During the various stages of mitosis, chromosomes adopt two major conformations: loose and tight. Before a cell divides, you cannot make out individual chromosomes in the nucleus because they are lank and gathered into an indistinctive clump. When a cell is about to divide, however, the lank chromosomes double their content and tighten up so as to adopt a more rigid shape while breaking away from one another. In this individualized, more rigid conformation, the mother cell can distribute them far more easily, and correctly, to the daughter cells. This is carried out by a structure known as the mitotic spindle – a sort of wonderfully evolved multichord mechanism, which can be compared to the chords of a violin gathered at each end. Each (doubled) chromosome is attached to one chord. As the mother

cell halves the chromosomes halve too, and each half is gently pulled into a nascent daughter cell.

How do chromosomes switch between their lank formless state and a more rigid condensed one? This puzzled scientists for years until they came up with a very elegant model that involves a protein known as Ki-67. Ki-67 is a large protein, with a high net electrical charge. It seems to have no particular 3D structure and spends most of its time unfolded like spaghetti. However, unlike spaghetti, Ki-67 has an amphiphilic molecular structure: its C-terminus is highly attracted to chromatin (what chromosomes are made of) while its N-terminus prefers the cytoplasm. The body of Ki-67 is made up of repeats that carry over 100 potential phosphorylation sites. Their phosphorylation causes Ki-67 to unfurl into a sort of tail, while dephosphorylation causes the tail to collapse. All in all, the structure of Ki-67 is very similar to that of surfactants, agents that are found at the boundaries of different phases, such as solid and liquid. Does Ki-67 actually behave like a surfactant? At the boundary of chromatin and cytoplasm?

This is the model that has been proposed. Ki-67 has no role in the internal structure of chromosomes, that is to say in their condensation as a cell is about to divide for instance. Ki-67's role is simply to keep condensed chromosomes apart during mitosis. It does this by forming a sort of repellent on the chromosomal surface. How? The C-terminus of Ki-67 binds to the chromosome while its extended N-terminus juts out into the cytoplasm. Consider the fact that an estimated 270,000 Ki-67 molecules bind to the surface of a mitotic (condensed) chromosome with an average spacing of about 69nm. Visually, this would look like a very hairy chromosome, whose whole surface is covered with a sort of brush-like arrangement of Ki-67. Since the body of each Ki-67 has a high – and identical – electrical charge, like magnets showing the same poles, two neighbouring chromosomes are repelled. In other words, they'll be kept separated from one another. This is how surfactants behave too.

Now, once the individual chromosomes have been dispersed in the daughter cells, they readopt their clumped state in a newly formed nucleus. What happens to the brush-like repellent on their surface? And to Ki-67? It would be natural to assume that since Ki-67 is not needed anymore, it is probably degraded. Scientists suggest something else. In its 'repellent' state, Ki-67 is highly phosphorylated which lends the protein a highly negative charge. At the end of mitosis, the body of Ki-67 is stripped naked as it is dephosphorylated thus giving it a highly positive charge. This might attract negatively-charged stretches of nucleotides known as ribosomal RNA (rRNA) that are found in a cell's nucleus. rRNA acts like a kind of glue as it binds to the 'collapsed tails' of Ki-67, concomitantly bridging neighbouring chromosomes. This is how the temporarily individualized chromosomes stick to one another and cluster in the newly formed nucleus.

So phosphorylation and dephosphorylation of Ki-67 would be the key to chromosome individualization and clustering, respectively. However, the role of phosphorylation in this process still has to be demonstrated. Likewise, there is currently no evidence of rRNA actually binding to Ki-67. For years, Ki-67 has been used both as a marker for cell proliferation and to assess the growth of tumour cells in cancer diagnostics. This was until researchers realised that the protein's role was probably less in cell proliferation *per se* than in the formation of two opposing structural states of chromosomes as a cell divides. Until evidence proves otherwise, what has been described above remains a model – but it certainly is a very elegant one, and demonstrates how powerful computational models can be, and how they are a researcher's precious ally.

Cross-references to UniProt

Proliferation marker protein Ki-67, *Homo sapiens* (Human) : P46013

References

1. Hernandez-Armendariz A., Sorichetti V., Hayashi Y., *et al.*
   A liquid-like coat mediates chromosome clustering during mitotic exit
   Molecular Cell 84: 3254-3270(2024)
   PMID: 39153474

2. Cuylen S., Blaukopf C., Politi A.Z., *et al.*
   Ki-67 acts as a biological surfactant to disperse mitotic chromosomes
   Nature 535: 308-312(2016)
   PMID: 27362226