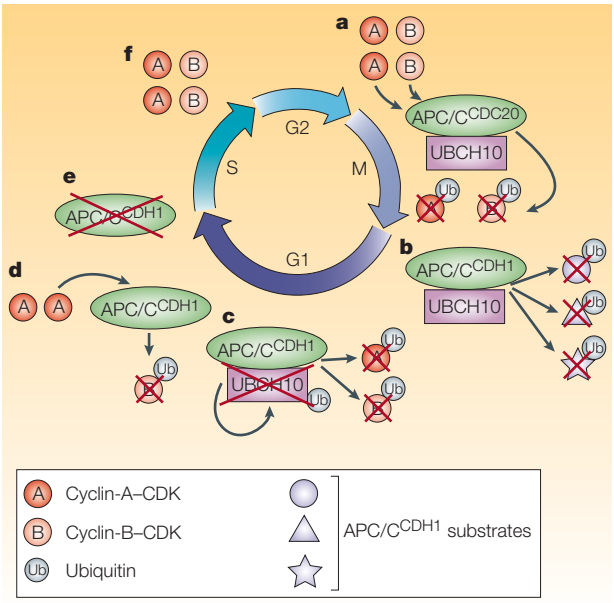
CELL CYCLE

What goes around…



The transition from mitosis (M phase) to G1 phase is controlled by complex interactions between two key components of the cell-cycle machinery, the anaphase-promoting complex/cyclosome (APC/C) and cyclin A. The APC/C is a multisubunit ubiquitin ligase that controls sister-chromatid separation and triggers mitotic exit through the ubiquitin-mediated degradation of proteins (including, in M phase, cyclin A). Cyclin A is an activator of the cell-cycle regulators CDK1 and CDK2 and, in late G1 phase, is also responsible for APC/C inactivation.

How cyclin A can re-accumulate in the presence of active APC/C during G1, and then inactivate the APC/C to allow the re-accumulation of other mitotic cyclins during the DNA synthesis (S) and G2 phases, has been a puzzle. In *Nature*, Michael Rape and Marc Kirschner now report that they have solved the mystery, by studying the effects that different components and regulators of the APC/C have on the degradation of cyclin A.

Initially, using extracts from synchronized HeLa cells, the authors showed that, whereas other APC/C substrates were consistently degraded throughout G1 phase, cyclin-A degradation gradually became blocked as cells progressed through G1. The degradation of cyclin A relied specifically on the presence of a threshold level of the ubiquitin-conjugating enzyme (E2) UBCH10, whereas other APC/C substrates were still degraded in the presence of another E2, UBCH5. In keeping with this, the authors showed that the level of UBCH10 (but not UBCH5) fluctuated during the cell cycle, remaining high during cyclin-A degradation and staying low when cyclin A was stable.

Intriguingly, it was then found that UBCH10 was autoubiquitylated before also being degraded by APC/C^{CDH1} towards the end of G1. However, this process was hampered both by the slow rate of UBCH10 autoubiquitylation and by the presence of other APC/C substrates. In fact, the authors showed that, as long as other APC/C substrates are present in G1, UBCH10 preferentially ubiquitylates them, rather than itself, which effectively allows UBCH10 to orchestrate the timing of cyclin-A

NUCLEAR TRANSPORT

Details of the doorway

The nuclear pore complex (NPC) functions as a doorway for the exchange of macromolecules between the nucleus and the cytoplasm, and a detailed view of this doorway is now revealed by Baumeister, Medalia and colleagues in *Science*.

The size of NPCs makes their structural characterization difficult, and previous structural studies have used isolated or detergent-extracted NPCs, which are susceptible to the loss of certain components and cargo. So, to get a more true-to-life view, Baumeister, Medalia and co-workers applied cryo-electron tomography (cryo-ET) to whole, transport-active *Dictyostelium discoideum* nuclei.

From the resulting tomograms, they extracted 267 volumes that contained an NPC and used averaging procedures to obtain an 8–9-nm-resolution, three-dimensional density map. In this map, the basic structural features of NPCs could be discerned: that is, eight cytoplasmic filaments that are attached to the cytoplasmic ring; the central, lumenal spoke ring; the central plug/transporter (CP/T) in the central channel; and the so-called nuclear basket (the nuclear ring and the distal ring that are connected by nuclear filaments).

However, the authors also noted several novel features — for example, the nuclear and cytoplasmic filaments appeared more bent and delicate than was previously reported, and the complex was less elongated along the nucleocytoplasmic axis. Furthermore, they found that the size, shape and position of the CP/T in the NPCs varied. The composition and role of the CP/T have remained unclear, but this observation indicates that it is composed, at least in part, of cargo complexes that are arrested in transit.

After further analysis, Baumeister, Medalia and colleagues noted that the NPCs were in one of two preferred states. In NPCs of the 'cytoplasmic-filament' class, the CP/T is in the plane of the cytoplasmic ring. The cytoplasmic filaments are in a defined orientation and are connected to the CP/T by an elongated density. This structure therefore seems to show cytoplasmic filaments in a cargo-bound state. By contrast, in NPCs of the 'lumenal-ring' class, the CP/T is in the plane of the lumenal spoke ring, and only the base of the cytoplasmic filaments can be seen. This structure therefore seems to show cytoplasmic filaments that have disengaged cargo and are free to move. Further differences in the organization of the lumenal spokes and the appearance of the nuclear basket could also be discerned between the NPC classes.

So, this work has shown that the CP/T is essentially cargo in transit and that NPCs undergo significant structural rearrangements during cargo transit. In addition, although many questions remain, it has shown that "…the application of cryo-ET to transport-competent, intact nuclei holds great potential for a structural dissection of the key steps involved."

Rachel Smallridge

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WEB SITE
Wolfgang Baumeister's laboratory:
http://www.biochem.mpg.de/baumeister/

accumulation through its own, carefully timed, self destruction.

So, Rape and Kirschner have identified what they suggest is the 'self-perpetuating oscillator' that pushes the cell cycle through its phases (see figure). Accumulation of cyclin-A–CDK1 and cyclin-B–CDK1 promotes entry into mitosis and activation of APC/C^{CDC20}, which, in turn, induces degradation of the mitotic cyclins and mitotic exit (a). APC/C acquires the CDH1 adaptor in G1 and degrades the remaining APC/C^{CDH1} substrates (b), which facilitates UBCH10 autoubiquitylation and degradation (c). Cyclin A then re-accumulates (d) and completely inactivates APC/C^{CDH1} (e), thereby initiating entry into S phase and allowing further mitotic-cyclin accumulation (f). So, as the saying goes: what goes around comes around…

Lesley Cunliffe

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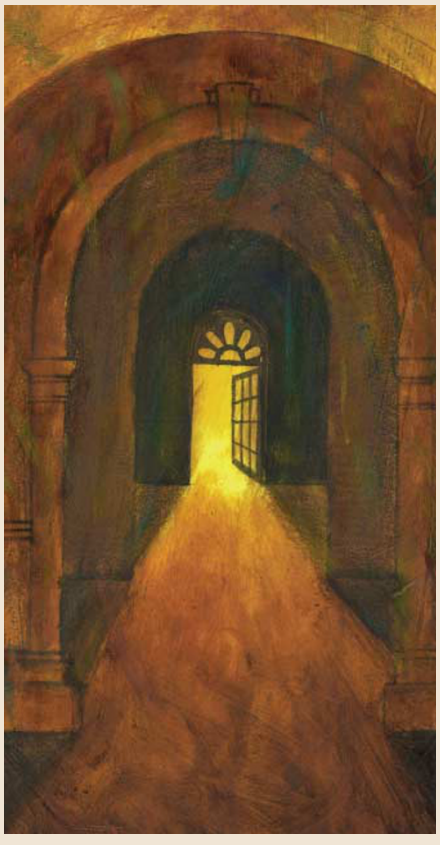

TRANSCRIPTION

Nuclear actin muscles in

The physiological significance of actin in the nucleus has been questioned in the past. However, several recent studies have implicated actin in RNA polymerase (Pol) II transcription. And, two papers now report that actin is also required for Pol I and Pol III transcription.

Given that actin usually works in conjunction with myosin motor proteins, and that a type of myosin I — NMI — has been shown to be involved in Pol II transcription, Ingrid Grummt and colleagues investigated the role of both actin and NMI in Pol I transcription, as reported in *Nature Cell Biology*. They found that depleting or inhibiting actin or NMI decreased Pol I transcription *in vivo*, thereby indicating that both proteins are involved in this process.

In vitro Pol I transcription assays with either naked DNA or pre-assembled chromatin required both actin and NMI. By contrast, the formation of short, trinucleotide transcripts in a so-called 'abortive transcription assay' was unaffected by treatment with anti-actin antibodies. So, actin and NMI have a direct role in ribosomal DNA transcription, rather than enhancing transcription through a chromatin-remodelling effect, and actin seems to function in a post-initiation event.

Immunoprecipitation experiments showed that both actin and NMI associated with the Pol I machinery. The fraction of Pol I that is associated with TIFIA, a basal transcription-initiation factor, is transcription competent. Interestingly, whereas actin was associated with the bulk Pol I population, NMI bound only to TIFIA-containing Pol I. In addition, the association of NMI with TIFIA-containing Pol I depended on the phosphorylation of TIFIA on Ser649 — a modification that is known to be required for transcription-initiation-complex formation.

Using chromatin immunoprecipitation (ChIP) assays, Grummt and co-workers showed that NMI associated with the rDNA promoter but not the coding region, whereas actin associated with both the promoter and the coding region. Together, these data indicated that actin and NMI have distinct roles in Pol I transcription — NMI functions in the assembly of Pol I transcription-initiation complexes, whereas actin functions in a post-initiation event, possibly transcription elongation.

Reporting in *Genes & Development*, Nouria Hernandez and colleagues found that highly purified Pol III contains β-actin. Interestingly, two of the Pol III subunits that were found to interact with β-actin are common to all three RNA polymerases. ChIP analysis indicated that β-actin localized close to the promoter region of an actively transcribed U6 gene *in vivo*, which indicates that β-actin might function in the transcription process.

Immunoprecipitated Pol III from cells that had been treated with a DNA-damaging agent lacked β-actin. Also, both Pol III and β-actin were largely dissociated from the U6 promoter in these cells, and Pol III that was purified from treated cells was inactive in an *in vitro* transcription assay. However, transcription could be reconstituted by adding β-actin and the CK2 kinase (which phosphorylates Pol III) to inactive Pol III.

So, the two studies clearly show a direct role for actin in Pol I and Pol III transcription and, given previous findings, the two groups propose that actin is required for the function of all three RNA polymerases.

Arianne Heinrichs

References and links

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