The nucleolus: the magician's hat for cell cycle tricks

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The nucleolus, for decades considered a ribosome factory and site for ribosomal RNA synthesis and processing, has recently acquired new fame. Analyses of proteins important for cell-cycle regulation have shown that this organelle is used to sequester proteins, thereby inhibiting their activity.

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Abbreviations
APC   anaphase promoting complex
CDK   cyclin-dependent kinase
SCF   Skp1–cullin–F-box protein

Introduction

Because the nucleolus is easily visualized by light microscopy, it was one of the first intracellular structures described [1,2]. It occupies a considerable portion of the nucleus, but its size varies greatly depending on the species, cell type and physiological state. In the 1940s cytologists learned that the nucleolus contains RNA and proteins [3]. In the1960s it was determined to be a 'ribosome factory' [3]. In the 1990s it is considered 'the plurifunctional nucleolus', because this organelle was found to participate in the biosynthesis and processing of RNA components of ribo-protein complexes [4]. How will it be portrayed in this new millennium? Recent studies regarding proteins involved in eukaryotic cell-cycle regulation suggest that the nucleolus might function as a 'prison'. Sequestration in the nucleolus prevents proteins from reaching their targets in other regions of the cell.

The eukaryotic cell cycle can be viewed as an irreversible reiteration of a precisely timed sequence of events: G_1, S phase, G_2 and mitosis. During S phase, the genetic material is duplicated and, after a rest phase (G_2), it is segregated equally between the two daughter cells during mitosis. Cells then exit from mitosis and enter another resting phase, G_1. Surveillance mechanisms, also known as checkpoints, ensure that cells do not progress through the cell cycle when defects occur [5]. Checkpoints sense intracellular stresses such as DNA damage and mitotic-spindle defects and halt cell-cycle progression until the damage is repaired or, in higher eukaryotes, induce apoptosis.

Work over the past twenty years has shown that cyclin-dependent kinases (CDKs) and ubiquitin-dependent degradation of key cell-cycle regulators promote cell-cycle transitions. CDKs associated with different cyclins trigger entry into the cell cycle, S phase and mitosis [6]. A specialized ubiquitin-dependent proteolysis complex, called the SCF-dependent proteolysis machinery (SCF for Skp1–Cullin–F–box protein) also regulates entry into the cell cycle by degradation of CDK inhibitors [7–13]. Sister-chromatid separation is mediated by another ubiquitin-dependent proteolysis complex, the APC-dependent proteolysis machinery (APC for anaphase promoting complex), that degrades inhibitors of this process [14–17]. By degrading the regulatory cyclin subunit of CDKs the APC-dependent proteolysis machinery also participates in promoting the final cell-cycle transition: exit from mitosis [14–16,18].

In the last year, three cell-cycle regulators have been identified whose activity is regulated by sequestration in the nucleolus. Cdc14, a protein phosphatase critical for promoting exit from mitosis [19•,20•], is kept inactive in the nucleolus until the onset of anaphase, thereby preventing the premature onset of mitotic exit [21••–23••]. Mdm2, an inhibitor of the tumor suppressor protein p53 (p53 induces cell cycle arrest in response to DNA damage), is sequestered in the nucleolus in response to activation of the oncoprotein Myc or replicative senescence, allowing p53 to become active [24••,25••]. Pch2, a protein required for halting meiotic cell-cycle progression in response to recombination and chromosome synapsis defects, also localizes to the nucleolus [26••], suggesting a possible role for nucleolar proteins and the nucleolus in checkpoint signaling. The finding that Cdc14, Mdm2 and Pch2 are sequestered in the nucleolus points to a novel role for this organelle in regulating the activity of cell-cycle regulators which will be the focus of this review.

The nucleolus and regulation of Cdc14

Exit from mitosis is ultimately triggered by inactivation of the CDKs that promote entry into mitosis [19•,20•]. In the budding yeast *Saccharomyces cerevisiae*, the phosphatase Cdc14 plays a critical role in promoting inactivation of mitotic kinases. By dephosphorylating an activator (Cdh1/Hct1) of the APC, Cdc14 induces degradation of these cyclins and thus inactivation of mitotic kinases [19•,20•,27]. Cdc14 also promotes accumulation of an inhibitor of mitotic kinases, Sic1, further ensuring that these kinases are inactivated at the end of mitosis [19•].

Immunolocalization studies revealed that Cdc14 is localized in the nucleolus during G_1, S phase, G_2 and metaphase. During anaphase Cdc14 spreads to the nucleus and, to some extent, the cytoplasm, where it dephosphorylates its targets. Biochemical purification of Cdc14-associated factors and a two-hybrid screen identified the nucleolar protein Cfi1/Net1 as the protein responsible for anchoring Cdc14 in the nucleolus

[21••–23••]. Cfi1/Net1 directly binds to rDNA, a DNA region encoding ribosomal RNAs, and anchors a number of proteins, including Cdc14 and Sir2, a protein involved in gene silencing [22••]. The regions in Cdc14 and Cfi1/Net1 responsible for nucleolar localization are not known.

The importance of sequestration of Cdc14 by Cfi1/Net1 in the nucleolus was revealed by the phenotypic analysis of cells lacking *CFI1/NET1*. In such cells, Cdc14 was present in the nucleus and cytoplasm throughout the cell cycle which causes cells to exit mitosis prematurely [21••,23••]. These findings led to the idea that Cfi1/Net1 sequesters Cdc14 in the nucleolus until the onset of anaphase, thereby preventing it from becoming active (Figure 1). In addition to inhibiting Cdc14 by sequestration, Cfi1/Net1 also appears to be a catalytic inhibitor of Cdc14 [21••]. How Cdc14 is liberated from Cfi1/Net1's deadly embrace during anaphase is a critical question that remains to be addressed. Cfi1/Net1 is a phosphoprotein, raising the possibility that phosphorylation is important for regulating the association between Cdc14 and Cfi1/Net1. Supporting this idea is the finding that release of Cdc14 from the nucleolus is controlled by a signal transduction pathway termed the mitotic exit pathway [28], which comprises at least four protein kinases. Clearly, the next challenge will be to determine whether and how phosphorylation affects the association between Cfi1/Net1 and Cdc14 and which protein kinase is responsible for phosphorylating Cfi1/Net1.

The nucleolus and regulation of Mdm2

In response to DNA damage a surveillance mechanism, known as the DNA damage checkpoint pathway, causes cells to arrest either in the G_1 or G_2 stage of the cell cycle [29]. In mammalian cells the p53 protein is a key component of the DNA damage checkpoint [5]. In response to DNA damage (and also other cellular stresses) p53 is transiently stabilized in the nucleus, where it becomes active as a transcription factor for genes that bring about cell-cycle arrest or apoptosis [30–33].

p53 protein levels and activity are under tight control. The oncogene *MDM2*, which itself is transcriptionally activated by p53, plays a pivotal role in regulating p53 protein levels and activity (Figure 2). Mdm2 is an inhibitor of p53, preventing its activity via at least two distinct mechanisms. First, Mdm2 binds to p53, thereby blocking its activity as a transcription factor [34,35]. Second, Mdm2 promotes p53 degradation by enhancing its export from the nucleus into the cytoplasm [36,37], and it may also act as a ubiquitin-protein ligase in the degradation of p53 [38]. Recently, it has been shown that in response to oncogenic signals the oncoprotein p19Arf stabilizes p53 [39•–42•]. In contrast to p53 stabilization brought about by γ-irradiation, p19Arf-induced stabilization of p53 is not due to phosphorylation of p53. Instead, p19Arf sequesters Mdm2 in the nucleolus, thereby preventing it from exporting p53 into the cytoplasm where it is degraded [24••,25••].

Figure 1
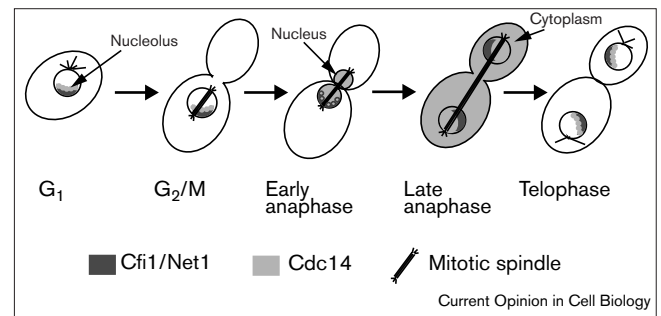
Localization of phosphatase Cdc14 during the cell cycle. During G_1, S phase and early mitosis, Cdc14 is sequestered in the nucleolus by Cfi1/Net1. After nuclear division has commenced, Cdc14 spreads into the nucleus and cytoplasm. It then dephosphorylates Cdh1 and Sic1 (not shown), thereby promoting the inactivation of mitotic kinases leading to exit from mitosis.

In murine cells p19Arf appears to exclusively localize to the nucleolus [24••,25••], which requires the carboxy-terminal region of p19Arf [24••]. Interestingly, overexpression of p19Arf leads to the recruitment of Mdm2, which is normally found in the nucleus and cytoplasm, into the nucleolus. p19Arf-dependent sequestration of Mdm2 was also observed in response to activation of the Myc oncogene and in cells undergoing replicative senescence [24••,25••]. The biological relevance of p19Arf-dependent sequestration of Mdm2 in the nucleolus was revealed by the analysis of a p19Arf mutant lacking the nucleolar localization sequence, a 106 amino acid region located in the carboxyl-terminal portion of the protein. This mutant is able to bind Mdm2, yet is unable to induce p53 stabilization and cell-cycle arrest, indicating that sequestration of Mdm2 by p19Arf in the nucleolus inhibits Mdm2's role in regulating p53 turnover [24••].

Analysis of the shuttling of Mdm2 between the nucleus and cytoplasm in heterokaryons showed that the longer it continued, the more Mdm2 protein localized with p19Arf in the nucleolus [25••]. This finding together with the observation that p19Arf is found exclusively in the nucleolus, suggests that Mdm2 moves from the nucleoplasm into the nucleolus, where it is sequestered by p19Arf [25••]. It raises the interesting possibility that Mdm2 is exported into the cytoplasm through the nucleolus. In this regard it is interesting to note that Mdm2's nuclear export signal is similar to that of TFIIIA, a protein that mediates nuclear export of the 5S rRNA [43,44]. Furthermore, Mdm2 binds to the ribosomal protein L5 and to the 5S rRNA and RNA sequences found in the 28S RNA of the large ribosomal subunit [37,45,46].

Whether sequestration of Mdm2 by p19Arf as a means of inhibiting Mdm2 function is conserved among mammals is unclear. In human cell lines, p19Arf inhibits Mdm2-mediated nuclear export of p53 but not by sequestering Mdm2 in

Figure 2

p53 degradation
by 26S proteasome
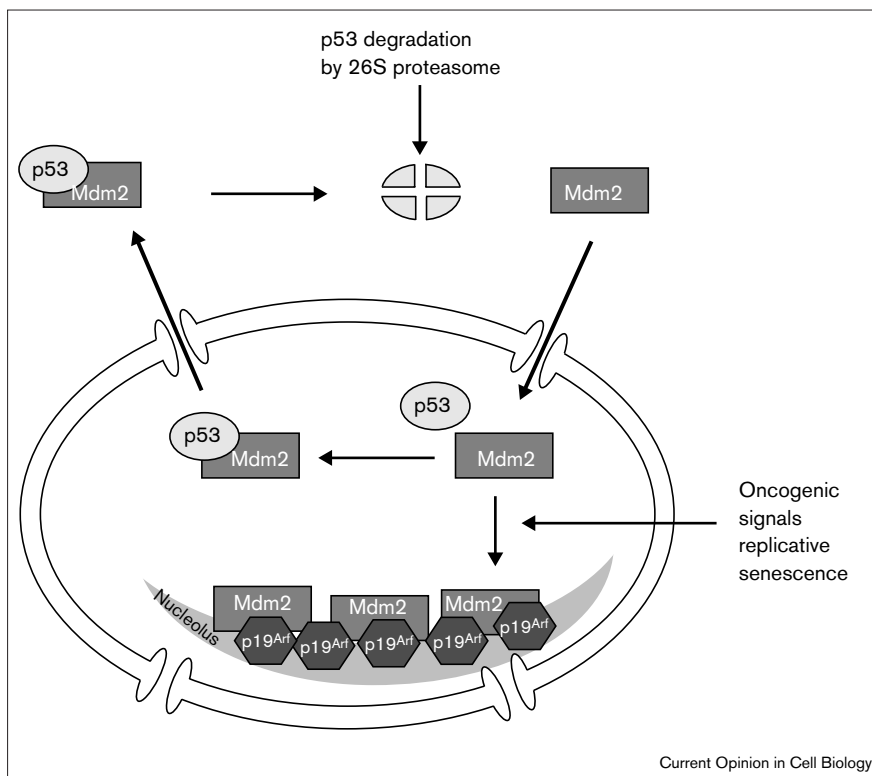
Regulation of Mdm2 and p53 by p19^{Arf}. In response to oncogenic signals and replicative senescence, p19^{Arf} sequesters Mdm2 in the nucleolus. This prevents Mdm2 from exporting p53 into the cytoplasm where it is degraded. Thereby, p19^{Arf} stabilizes p53, allowing it to induce cell-cycle arrest or apoptosis.

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the nucleolus [47•]. High levels of Mdm2 cause relocalization of p19^{Arf} from the nucleolus into the nucleoplasm where it forms nuclear bodies with Mdm2 and p53 [47•]. Thus, it appears that although Mdm2 sequestration in the nucleolus is important in murine cells for inhibition of Mdm2 activity, it is dispensable in human cells. However, we cannot exclude the possibility that differences in experimental conditions, such as the level of expression of different factors, account for the differences in the experimental outcome.

The role of the nucleolus in regulation of the pachytene checkpoint

Meiosis is a specialized cell cycle in which a single S phase is followed by two consecutive nuclear divisions [48,49]. During prophase of the first meiotic division homologous chromosomes undergo genetic recombination and synapsis. In both yeast and mammals mutants defective in recombination or formation of the synaptonemal complex, a proteinaceous structure responsible for synapsis, arrest in pachytene due to activation of the pachytene checkpoint [50–56].

A hunt for mutants defective in cell-cycle arrest in response to defects in synapsis in the budding yeast *S. cerevisiae* identified a meiosis-specific gene, *PCH2*, whose product localizes predominantly to the nucleolus [26••]. During pachytene when chromosomes are fully condensed and synapsed, most of Pch2 localizes to the nucleolus, a region

that does not undergo synapsis and recombination. A small pool of Pch2 is also found in a punctuate pattern along synapsed chromosomes. Localization of Pch2 to the nucleolus requires *SIR2* and, consistently, inactivation of *SIR2* also caused inactivation of the pachytene checkpoint. How Pch2 functions in the nucleolus to inhibit meiotic cell-cycle progression in response to defects in recombination or synapsis is at present unclear. The absence of or defects in synapsis could lead to accumulation of Pch2 (which otherwise localizes along paired homologs) in the nucleolus where it is recognized as 'non-nucleolar', causing the activation of the pachytene checkpoint. Alternatively, as *PCH2* is required for repression of recombination in the rDNA region, inactivation of *PCH2* could lead to increased recombination in the rDNA region, leading to activation of the pachytene checkpoint. Clearly, determining Pch2's function and the analysis of *pch2* mutants defective in nucleolar localization will be required to determine the role of nucleolar Pch2 in regulation of the pachytene checkpoint.

Conclusions

Work over the last year has revealed a new role for the nucleolus in regulating the activity of proteins involved in various aspects of cell-cycle progression. Sequestration in the nucleolus prevents proteins from reaching their targets in other cellular compartments. Whether more proteins exist that are regulated in this way remains to be determined. The existence of a sequence that drives nucleolar localization would help identify such proteins. However, although basic amino

acid stretches are required for nucleolar localization, recruitment into the nucleolus does not appear to be brought about by a common targeting signal. Rather, interactions with other proteins already present in the nucleolus recruit proteins into this compartment. This raises the interesting possibility that rDNA binding proteins exist that function as anchors or 'nucleolar receptors' for other proteins. Cfi1/Net1 could be such an anchor. Cfi1/Net1 not only sequesters Cdc14 in the nucleolus but also anchors Sir2 [22••]. As Pch2 localization to the nucleolus depends on Sir2 [26••], Cfi1/Net1 is probably also responsible for anchoring Pch2. Whether this involves direct binding of Cfi1/Net1 to Pch2 remains to be determined.

It is certainly puzzling that cells choose sequestration in the nucleolus as a means of inhibiting protein function. Perhaps the nucleolus is one of the few places in the cell where a protein, that functions in the nucleus and cytoplasm and whose activity needs to be tightly regulated can be locked away until it is required. Consistent with this idea is the finding that proteins present in the nucleolus are usually not found in the rest of the nucleus and *vice versa*. The advantage of sequestration in the nucleolus, as opposed to other organelles, is that they can, upon release, quickly reach their targets in the nucleus and cytoplasm. A second possibility is that these proteins have another part-time job in the nucleolus. Indeed, Cdc14 appears to participate in nucleolar segregation during mitosis [57] and Pch2 is required to prevent recombination in the rDNA region [26••]. Whether sequestration in the nucleolus is a means commonly used by the cell to inhibit the function of proteins remains to be determined. This will be the challenge for this millennium.

Acknowledgements

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