Published in final edited form as:

Nat Cell Biol. 1999 November ; 1(7): E175–E177. doi:10.1038/15611.

Saying a firm ‘no’ to DNA re-replication

J. Julian Blow and Tatyana A. Prokhorova

Abstract

Yeast cells exclude the DNA-replication-initiation factor Mcm4 from the nucleus during the S and G2 phases of the cell cycle, when active replication ‘origins’ must not reassemble. This exclusion is dependent on cyclin-dependent kinases and provides a general mechanism for preventing re-replication of DNA in a single cell cycle.

Sometimes when you say ‘no’, you have to mean it. This is certainly the case with the re-initiation of replication forks on chromosomal DNA, because once a replication origin has been initiated, it must absolutely not initiate replication again in the current cell cycle. If it did, the result would be the permanent amplification of DNA in that region, with likely disastrous consequences. On page xxx of this issue, Labib et al.¹ characterise one of the ways in which yeast cells may ensure that this “no” is absolute - by physically separating one of the key initiation activities, Mcm4, from template DNA. Yeast do this by exporting free Mcm4 from the nucleus at stages of the cell cycle when it is no longer needed. The export process is dependent on Cyclin-dependent kinase (Cdk), which in addition to being required for the initiation of replication may thereby also prevent the re-replication of DNA.

The Mcm family (also known as the MCM/P1 family) consists of 6 closely-related proteins that are all assembled onto replication origins during late mitosis and G1 to form a “pre-replicative complex” (pre-RC)². This assembly reaction results in the origin becoming “licensed” for DNA replication in the subsequent S phase, and depends on two other origin-binding proteins, ORC and Cdc6. In the frog *Xenopus laevis*, a third activity termed RLF-B is required³, and another general necessary condition is the absence of Cdks which inhibit licensing. The Mcm proteins are removed from the DNA as it is replicated and this is a primary reason why chromosomal DNA replicates only once in a single cell cycle. It is therefore crucial that Mcm proteins are never re-assembled on replicated DNA. This could be achieved by the inactivation of one or more components of the licensing reaction. Alternatively, as was originally proposed to define the idea of replication “licensing”, an essential component could be excluded from the nucleus⁴. Hennessy et al (1990) have shown that in the yeast *Saccharomyces cerevisiae*, the Mcm5 protein changes its subcellular distribution during the cell cycle, being nuclear in late mitosis and G1, and cytoplasmic during S, G2 and early mitosis⁵. This observation first suggested that Mcm proteins were involved in replication licensing, though the mechanism remained obscure.

Labib et al have investigated the subcellular localisation of Mcm proteins using Mcm4 fused to Green Fluorescence Protein (GFP), allowing the protein to be directly visualised going about its normal business within live yeast cells. The expression of Cdc6 (which is required for Mcms to bind DNA) was artificially controlled so that the localisation of Mcm4-GFP could be examined independently of its DNA binding. Labib et al. observed that free Mcm4-GFP (i.e. Mcm4-GFP not bound to DNA) is found in the nucleus only in the absence of Cdk activity, and once Cdks are activated, free Mcm4-GFP is exported into the cytoplasm. In a normal cell cycle, Mcm4 therefore remains cytoplasmic during S phase, G2 and early mitosis whilst Cdks are active - times when the licensing of replication origins does not occur (Fig 1A). All of the 6 Mcm proteins form complexes that are required for licensing, so they probably all act in concert. It is likely that only two of the 6 Mcm proteins (Mcms 2 and

3) contain nuclear localisation sequences and are responsible for co-transporting the others ^{6, 7}.

This effect may play a role in preventing re-replication of DNA in a single cell cycle. Previous work in fission yeast has shown that inhibition of Cdk activity in G2 causes the cell to return to a G1-like state where re-licensing occurs. When Cdk activity is subsequently restored, the cell performs an extra S phase and re-replicates its DNA ⁸. Consistent with this, Labib et al show that when Cdk activity is inhibited in G2 cells, Mcm4-GFP translocates back into the nucleus, where it is potentially available for re-assembly on DNA. But is nuclear exclusion of Mcms really important in preventing re-replication? Since re-licensing of even a single origin is potentially lethal, there might well be more than one mechanism to prevent it. In yeast, Cdks are known to induce the degradation of Cdc6, which by itself would block pre-RC formation. However, supplying cells with a non-degradable version of Cdc6 does not allow pre-RCs to form in G2, so there must be at least one other block to re-replication. A plausible mechanism for this additional block would be Cdk-induced exclusion of Mcms from the nucleus, separating them from the template DNA. It would be interesting to know whether a cell expressing both a constitutively nuclear form of Mcms and a non-degradable Cdc6 would spontaneously re-replicate its DNA, or whether further redundant mechanisms operate.

In *S. cerevisiae*, Cdk activity is driven by two different classes of cyclin: Clns (G1 cyclins) which are active in late G1 and prepare cells for S phase, and Clbs (B-type cyclins) which are activated from the very start of S phase through to mitosis, and which induce the initiation of replication. Labib et al ¹ report a curious difference in the effect of Cln- and Clb-driven Cdk activity on the subcellular localisation of Mcm4. Both Clns and Clbs cause Mcm4-GFP to accumulate in the cytoplasm in the absence of Cdc6 (conditions where Mcm4 cannot bind to DNA) (Fig 1B, left), whereas in the presence of Cdc6 only Clbs, but not Clns, prevent the appearance of nuclear Mcm4 (Fig 1B, right). Labib et al suggest that Clns and Clbs affect the subcellular distribution of Mcm4 in different ways: Clbs block Mcm4 nuclear import (Fig 1Bii) whilst Clns may simply make Mcm4 nuclear export exceed its import but allow the protein to shuttle between nucleus and cytoplasm (Fig 1Bi). Therefore in the presence of Clns, Mcm4 passing through the nucleus could be recruited onto pre-RCs by Cdc6. An alternative interpretation is that both Clns and Clbs affect the subcellular distribution of Mcm4 in similar ways by making export exceed import, but that Clbs (but not Clns) inhibit the assembly of Mcm4 onto DNA by a separate pathway (Fig 1Ciii). Consistent with this, in the normal cell cycle Clns are present in late G1 when pre-RC assembly does not need to be inhibited, whereas Clbs are present in S and G2 when the re-assembly of pre-RCs would be harmful. In order to determine which explanation is correct, more needs to be known about how the localisation of Mcm proteins is controlled. Do they shuttle between nucleus and cytoplasm during G2? Do Cdks affect the rate of import, the rate of export, or both? Is the effect mediated by direct phosphorylation of Mcm proteins by Cdks, perhaps on nuclear localisation sequences as occurs on other proteins? In favour of this, Mcm3 is known to be phosphorylated in G2 ⁹ and have potential Cdk phosphorylation sites close to its nuclear localisation sequence ⁷.

Although these studies were performed in yeast, they have clear implications for the control of DNA replication in higher eukaryotes. Unlike yeast, metazoan Mcm proteins do not change their subcellular distribution during the cell cycle, being exclusively nuclear. Despite this difference, higher eukaryotes do seem to regulate replication licensing and the formation of pre-RCs by selectively partitioning proteins between nucleus and cytoplasm (Fig 2). The cell cycle of the *Xenopus* early embryo has no appreciable G1 period, and the re-licensing of replicated DNA occurs only in late mitosis and is absolutely dependent on the breakdown of the nuclear envelope ⁴. The exclusion of an essential licensing component by the nuclear

envelope would provide a simple way of preventing re-replication of DNA in a single cell cycle. It is currently unknown which factor is excluded, since Mcms and Cdc6 appear to remain nuclear throughout interphase, though RLF-B remains a good candidate³. Yet another variation is seen in mammalian cells, where Cdc6, rather than Mcms, appears to be regulated by subcellular localisation¹⁰. In G1 phase, mammalian Cdc6 is nuclear, but becomes cytoplasmic in S phase, where it remains until the following G1. Although the mechanism causing this re-localisation is currently unknown, a role for Cdks in nuclear exclusion is plausible. Although yeast, *Xenopus* and mammals have apparently chosen different protein targets, all three seem to use exclusion of essential initiation factors from the nucleus to prevent re-replication of DNA in a single cell cycle. This physical separation of a protein from its substrate may provide a robust way to make sure that “no” means “absolutely no”.

References

1. Labib K, Diffley JFX, Kearsley SE. Nat. Cell Biol. 1999
2. Tye BK. Ann. Rev. Biochem. 1999; 68:649–686. [PubMed: 10872463]
3. Tada S, Chong JPI, Mahbubani HM, Blow JJ. Curr. Biol. 1999; 9:211–214. [PubMed: 10074431]
4. Blow JJ, Laskey RA. Nature. 1988; 332:546–548. [PubMed: 3357511]
5. Hennessy KM, Clark CD, Botstein D. Genes Dev. 1990; 4:2252–2263. [PubMed: 2279699]
6. Kimura H, Ohtomo T, Yamaguchi M, Ishii A, Sugimoto K. Genes Cells. 1996; 1:977–93. [PubMed: 9077461]
7. Young MR, Suzuki K, Yan H, Gibson S, Tye BK. Genes Cells. 1997; 2:631–43. [PubMed: 9427284]
8. Broek D, Bartlett R, Crawford K, Nurse P. Nature. 1991; 349:388–393. [PubMed: 1992340]
9. Kimura H, Nozaki N, Sugimoto K. EMBO J. 1994; 13:4311–4320. [PubMed: 7925275]
10. Saha P, et al. Mol. Cell. Biol. 1998; 18:2758–2767. [PubMed: 9566895]

Figure 1.

Cdk-dependent localisation of Mcms. **A.** Cell cycle changes in Mcm4 localisation during the yeast cell cycle. During early G1 when Cdk activity is absent, Mcms enter the nucleus and bind to DNA. During S phase, Clb-Cdks induce the initiation of replication, displacing bound Mcm protein. Cdk activity further excludes Mcms from the nucleus. **B.** Possible mechanisms for Cdk-driven re-localisation of Mcms. Left: wild-type cells; right: cells expressing stabilised Cdc6. **Bi.** Cln-Cdks cause net export of Mcms from the nucleus, though Mcm shuttling still occurs so that if Cdc6 is present (right), Mcms passing through the nucleus bind to DNA. **Bii.** Clb-Cdks block Mcm nuclear import, so that even if stabilised Cdc6 is present, no Mcms are available to bind DNA; or **Biii** Clb-Cdk causes net export of

Mcms but allows shuttling to occur; Clb-Cdk also blocks origin licensing so that even if stabilised Cdc6 is present, Mcms passing through the nucleus cannot bind to DNA.

Figure 2. Re-distribution of pre-RC components between nucleus and cytoplasm during S/G2 phases of cell cycle in different organisms. Yeast export Mcms and degrade Cdc6, *Xenopus* excludes another factor, possibly RLF-B, whilst mammalian cells export Cdc6.