For 60th birthday of BBRC: DNA replication factors outside S phase

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DNA replication is one of the most fundamental processes for cell growth. Since the proposal of semi-conservative DNA replication by Watson-Crick and discovery of an enzyme responsible for this process by Kornberg, studies on DNA replication for the past 67 years revealed the involvement of many proteins for regulated execution of DNA replication [1].

Among them, ORC (Origin Recognition Complex), Cdc6, MCM (minichromosome maintenance), and Cdt1 are essential for forming pre-RC, a protein complex required for initiation of DNA replication, on chromatin during G1 phase. Mutations in these genes generally lead to reduced licensing (less pre-RC on the chromatin), resulting in inefficient S phase progression or increased sensitivity to replication stress [2,3].

Extra-replication functions of the pre-RC factors have been indicated. Roles of ORC in transcription silencing were reported shortly after identification of ORC in budding yeast [4,5]. Orc6 subunit of the ORC complex was shown to localize at kinetochores and to the midbody before cytokinesis, and Orc6 depletion led to aberrant nuclear division and mitosis [6,7].

In BBRC 456 (2015) 763–767, Deog Su Hwang's group at Seoul National University reported that Cdc6, an evolutionally conserved replication factor essential for pre-RC formation, is localized to centrosomes during S-G2 phase [8]. The group identified a 56 amino acid segment of Cdc6 which is sufficient for centrosome localization. Similar finding was reported by other group later in the same year [9]. This is an important paper that clearly indicated an important function of Cdc6 in regulation of the centrosome functions. Further studies showed Cdc6 negatively regulates the microtubule-organizing activity of the centrosome by inhibiting the recruitment of pericentriolar material (PCM) proteins to the centrosome [10,11]. Furthermore, Cdc6 was shown to inhibit over-duplication of centrosomes [11,12]. Orc1 was reported to control centrosome copy

number [13].

Orc2 is known to localize to centrosomes [14], and the partner of Cdt1, Geminin, was also reported to associate with centrosomes [15]. The finding by Hwang added Cdc6 to the list of centrosome-associated replication factors. Other groups also reported association of Mcm components to centrosomes [16,17]. Further functional analyses of the centrosome localization of replication factors led to a general conclusion that pre-RC factors negatively regulate centrosome biogenesis.

These findings revealed unexpected roles of pre-RC factors in regulation of centrosome duplication, in addition to its roles in genome duplication. Replication of DNA and duplication of centrosomes are two essential events for cell proliferation, that need to occur “once and only once” during cell cycle in a coordinated manner. pre-RC components play pivotal roles in ensuring ordered and controlled execution of DNA replication and mitosis, firstly by assembly of replication complexes in a proper timing and secondly by defining the timing of centrosome duplication and prevention of its overduplication.

The diverse functions of pre-RC factors in cell cycle progression predict existence of diseases caused by their mutations. Indeed, an autosomal recessive Meier-Gorlin syndrome (MGS) [18-21], an entity of Microcephalic primordial dwarfism, has been shown to be caused by mutations in preRC components including Orc1, Orc4, Orc6, Cdt1 and Cdc6 [22]. In view of the multiple roles of the preRC factors during cell cycle, as described above, mutations in these genes would affect cell proliferation and survival by affecting DNA replication and nuclear/ cell divisions as well as coupling of these processes.

In addition to the roles of pre-RC factors in DNA replication and chromosome separation, a striking defect in the rate of formation of

primary cilia, a modified centriole, was detected in mutants of preRC components. It was also proposed that reduced efficiency in forming cilia could be responsible for clinical features of MGS [23].

Factors participating the processes of DNA replication have been largely identified, and the mechanisms of how they regulate different steps of DNA replication are being elucidated at a rapid rate. However, the functional analyses of replication factors on cellular and an animal level will continue to reveal their unexpected functions. Studies using genetically manipulated model species carrying specific forms of mutations or those bearing a tissue/ organ-specific gene knockout are being awaited to fully understand the biological functions of replication factors.

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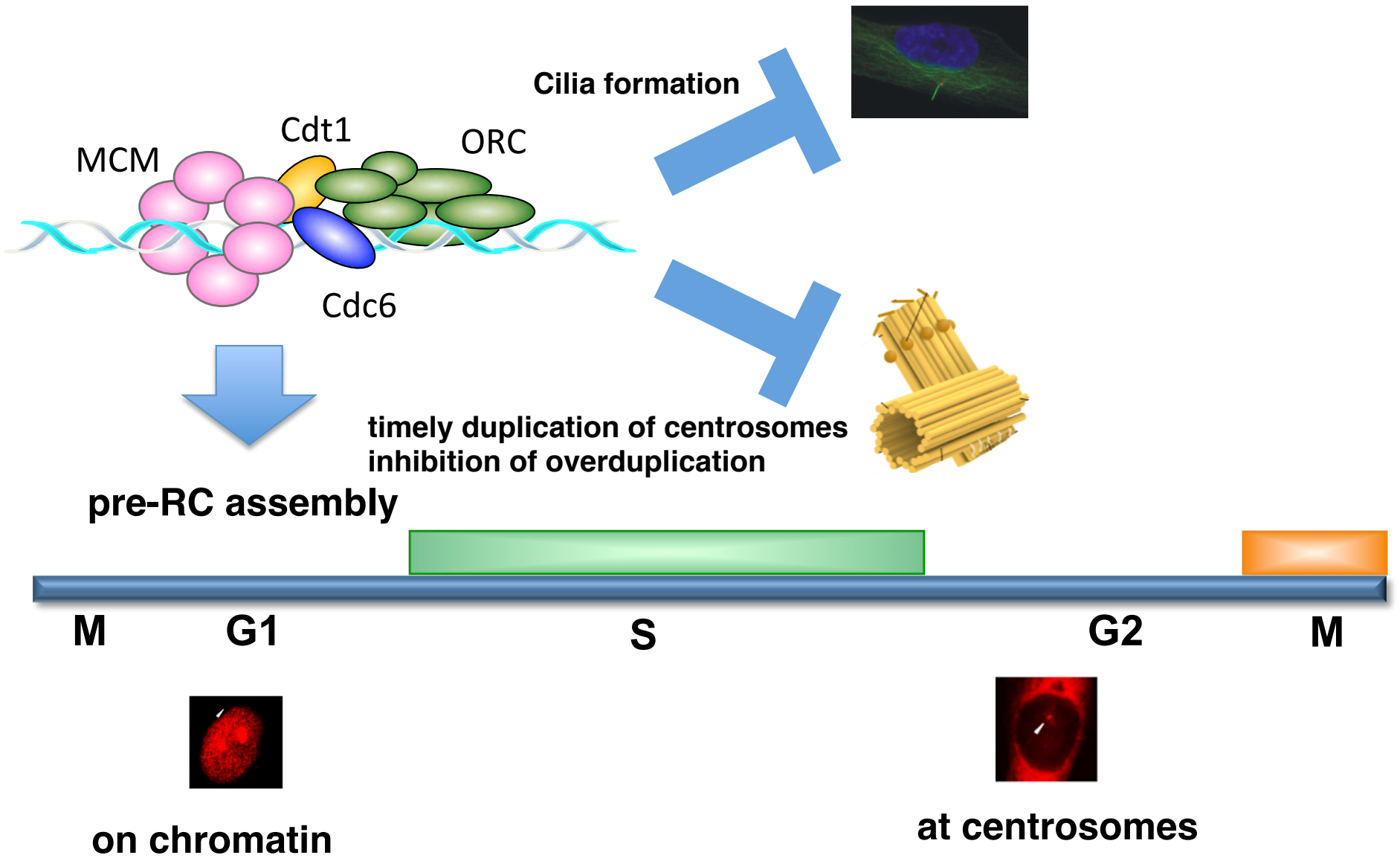
Legends to figure

During the G1 phase of the cell cycle, pre-RC factors play essential roles in assembly of a protein complex that is prerequisite for initiation of DNA replication. Pre-RC formation is strictly prohibited once S phase is initiated. Pre-RC factors binds to centrosomes and inhibits overduplication of centrosomes. Its role is generally inhibitory for the centrosome functions, and it may be required for timely and regulated duplication of centrosomes. Pre-RC factors may also inhibit formation of cilia, The two lower panels are cell images from ref. 8 (red, Cdc6 protein), and the upper panel is from ref. 23 (green, acetylated tubulin [entire cilia]; red, γ-tubulin antibodies [the basal body]).



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