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The Activating Kinases Cdk2 And Cdc7 In Oxygen Dependent Regulation Of Mammalian DNA Replication

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ABSTRACT

In eukaryotes, the initiation of DNA replication is reversibly arrested by hypoxia at hypoxic pre-initiation state and which enters in to burst of initiation by reoxygenation. The hypoxic pre-initiation state appears similar to pre-replicative complex (pre-RC) in eukaryotes. The pre-RC is switched for replicon initiation by the activating kinases Cdk2 and Cdc7. In O_2 dependent regulation of replicon initiation, Cdk2 activity is reported to be dispensable for the activation of hypoxic pre-initiation state by reoxygenation after several hours hypoxia. The phosphorylation of the second activating kinase Cdc7, the highest interaction with its substrate MCM2 and, the following phosphorylation and dissociation of MCM2 from the chromatin after reoxygenation of hypoxically suppressed replication provide a new pathway, which may be a possible pathway to release hypoxically arrested replicon initiation in course of reoxygenation.

KEYWORDS

Hypoxia;
Reoxygenation;
Cdk2;
Cdc7;
MCM2;
T24 cells;
Replication;
Chromatin.

INTRODUCTION

The complete duplication of the genetic material by DNA replication is an universal feature of the cell division cycle in all organisms. The initiation of DNA replication is a strictly regulated process as the genome has to be replicated once, but only once per cell cycle. In eukaryotes, the initiation of DNA replication is cell cycle regulated, requiring the stepwise assembly of a pre-replicative complex(pre-RC) in G1-phase. On completion of pre-RC assembly the chromosomes are licensed for replication during S-phase by activating kinase Cdk2 and Cdc7[1,2]. On the other hand, when the cells are subjected to hypoxia directly,

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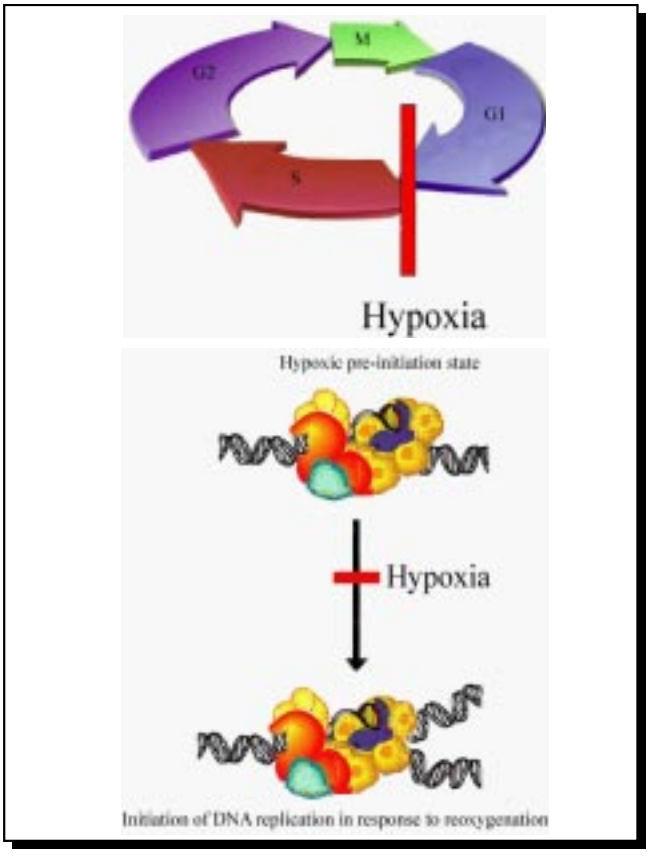
after restimulation with fresh medium, replicon initiation is reversibly arrested and which enters into synchronous wave of initiation by elevating the oxygen level[3-8]. This O_2-dependent replication control acts very directly on the replication apparatus itself and which has been shown so far in all types of cells examined in this respect during the past 20 years, ranging from cells replicating a virus(SV40) over a diversity of tumour cell lines to normal human primary explanted from umbilical cord vein(HUVEC) and nasal epithelium(HNEpC) (Probst, G., Probst, H., Gekeler, V., to be published). In case of mammals, especially in T24 cells, human bladder cancer cells, reoxygenation after several hours hypoxia initiates replicon in synchronous wave followed normal daughter strand by elongation and termination in the concerned, and normal further cycling of the cells succeeds[5,9,10]. Analyzing the replication proteins in T24 cells suggests that replicon arrest by hypoxia is situated very close before actual initiation. Reoxygenation induces the cellular changes of the replication proteins and results in burst of replicon initiation[5,9]. This mechanism is still largely unknown. On the other hand, this oxygen dependent regulation of replicon initiation is an important property of proliferating mammalian cells, serving basal function, such as protection against metabolic catastrophes during embryonic development or wound healing and in tumour growth[7]. This review focuses the role of activating kinases Cdk2 and Cdc7 in oxygen dependent regulation of DNA replication in mammals.

Hypoxia arrests replicon initiation at hypoxic pre-initiation state in mammals

DNA replication in eukaryotes is initiated by stepwise assembly of a pre-replicative complex (pre-RC) in G1-phase. Pre-RC is assembled through sequential binding of ORC, Cdc6, Cdt1 and MCM proteins at origins[1,11]. On completion of pre-RC assembly the chromosomes are licensed for replication during S-phase by cyclin-dependent kinase Cdk2 and the ASK/Cdc7 kinase[1,2]. On the other hand, a specialized elaborated starvation protocol accumulates T24 cells in G1 phase. Cells enter to S phase in response to medium renewal with fresh medium. This conversion of G1 phase to S phase is blocked, when starved T24 cells were incubated hypoxically directly after stimulation by medium renewal[5]. Here, the replication initiation is reversibly

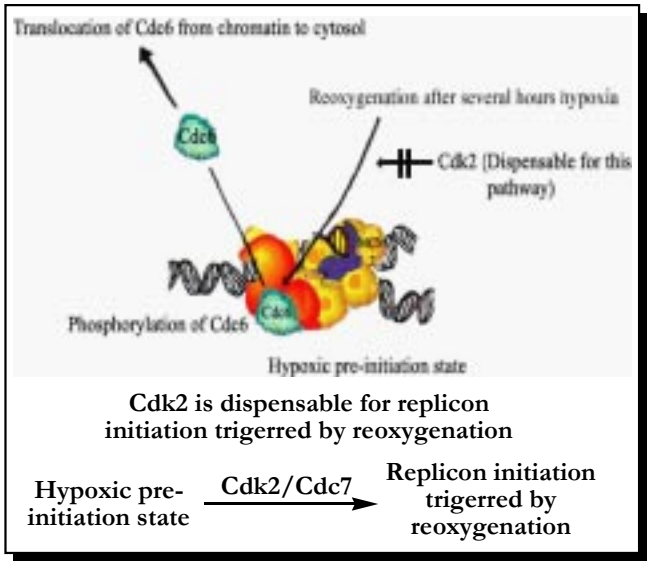


arrested. Reoxygenation after several hours hypoxia releases the block and cells enter to S phase and following successful cell cycle process. The study of analysing the changes of replication proteins suggests that hypoxia arrests replicon initiation at a specific state, 'hypoxic pre-initiation state', in which MCM2/MCM3 and Cdc6, as well as the activating kinases Cdk2 and Cdc7 become bound to chromatin already, thus enabling hypoxic cells to initiate as soon as the hypoxic suppression of replicon initiation is released[5,9,10]. This hypoxic pre-initiation state is similar to the pre-RC described by Bell and Dutta. In Eukaryotes, the pre-RC is activated for active DNA replication by two essential kinases Cdk2 and Cdc7[1,2]. In O_2 dependent regulation of DNA replication, reoxygenation triggers the switching process of hypoxic pre-initiation state to successive replicon initiation following elongation process[5]. Hypoxia interferes the switching process of hypoxic pre-initiation state to replicon initiation. To Understand the mechanism, the participation of activating kinases Cdk2 and Cdc7 in the activation of hypoxic pre-initiation state by reoxygenation is studied.

Cdk2 role in the activation of hypoxic pre-

initiation state

Cdk2 in association with cyclin E is known to be essential for the activation pre-RC to active replicon initiation. Protein phosphorylations by Cdk2 were suggested to be important for pre-RC assembly and activation[12]; for example, together with Cdk4, the phosphorylation of pRb and the subsequent release of E2F results in the transcription of essential factors of the replication initiation complex such as MCM and Cdc6[12,13]. Cdk2/Cyclin A mediated phosphorylation of Cdc6 is thought to induce its translocation from the nucleus to the cytoplasm in order to preventing reinitiation. The elevated levels of the Cdk2 inhibitor p27 and decreased activity of Cdk2 in conjunction with hypophosphorylated pRb have been shown to result in growth arrest[14]. In addition, Cdk4 can substitute for Cdk2 in pRb phosphorylation, and proliferation of cancer cells that do not contain pRb, may be completely independent of Cdk2 or Cdk4 activity[15]. In T24 cells, Cdk2 is reported to bound chromatin in hypoxic and reoxygenated cells. The pRb is hypophosphorylated in hypoxic cells and remains hypophosphorylated following 30min reoxygenation[5,9]. As above mentioned Cdc6 is one of the substrate for Cdk2 and Cdc6 dissociates from chromatin through its phosphorylation after initiation burst triggered by reoxygenation of hypoxic cells. The assumption was Cdk2 could be a switch for hypoxic pre-initiation state to active replicon initiation in response to reoxygenation. The recent studies clearly show that Cdk2 is not essential for releasing hypoxic replicon arrest by elevating oxygen level. The phosphorylation and translocation of Cdc6 after reoxygenation is not affected by inhibiting the kinase activity of Cdk2[9]. The cellular changes of pRb in response to hypoxia as well as reoxygenation are not affected by inhibiting Cdk2 kinase activity. Furthermore, synthesis of PCNA and growing daughter strand DNA analyses show that inhibition of Cdk2 has no influence on initiation burst triggered by reoxygenation of hypoxic replicon arrested T24 cells[9]. The activating kinase Cdk2 is reported to be not essential for the activation of hypoxic pre-initiation state. The next activating kinase, Cdc7 comes in the suspicion in the oxygen dependent regulation of DNA replication.



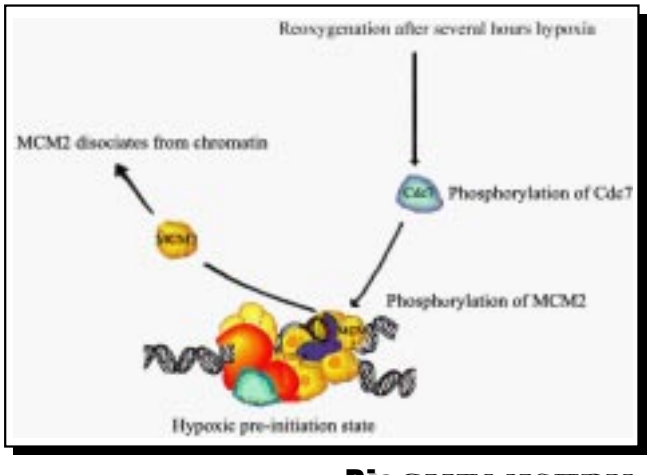
Cdk2 is dispensable for replicon initiation trigerred by reoxygenation

Hypoxic pre-initiation state → **Cdk2/Cdc7** → **Replicon initiation trigerred by reoxygenation**

Is Cdc7 kinase a possible switch for initiation burst triggered by reoxygenation?

Cdc7 is the next essential kinase for the activation of pre-RC to DNA replication in eukaryotes. Activation of Cdc7 requires association with the regulatory protein Dbf4 in S.cerevisiae, Dfp1/Him1 in S.pombe or ASK in mammals[16-20]. In eukaryotes, one of the conserved substrates of Cdc7 kinase is MCM2, an important component of MCM2-7 complex[1,2,21-23]. Biochemical studies suggest that the presence of MCM2 inhibits the helicase activity of the MCM4/6/7 complex[24,25]. The phosphorylation of MCM2 by Cdc7 is suggested to be a critical step in the initiation of DNA replication, eventually leading to release of MCM2 and resulting in activation of the MCM4/6/7 helicase activity[22,26-28]. In the activation of hypoxic pre-initiation state, reoxygenation induces the phosphorylation of Cdc7 in T24 cells[10].

However, Cdc7 is reported to be bound as unphosphorylated form in chromatin under hypoxic replicon arrest. Reoxygenation after several hours hypoxia induces the phosphorylation of Cdc7 and, also



results in phosphorylation and dissociation of MCM2 from the chromatin. An increase of interaction between Cdc7 and MCM2 after reoxygenation is also reported[10]. Thus, Cdc7 is a possible switch for reoxygenation to activate hypoxic pre-initiation state for active replicon initiation. Together, these results clearly suggest a pathway that reoxygenation activates Cdc7 kinase, thus results in phosphorylation and dissociation of MCM2 from the chromatin and which may result in the activation of MCM4/6/7 helicase[24,25]. This Reoxygenation-Cdc7 pathway may be a possible pathway to activate hypoxic pre-initiation state for active replication by reoxygenation.

CONCLUSION

The kinases, Cdk2 and Cdc7 are known to be essential for the activation pre-RC for active DNA replication in eukaryotes. The several hours hypoxia arrests replicon initiation reversibly at hypoxic pre-initiation state. The hypoxic pre-initiation state is almost similar to pre-RC in mammalian cells. The reoxygenation after several hours hypoxia activates the hypoxic pre-initiation state for replicon initiation burst. The suspicion was reoxygenation may activate hypoxic pre-initiation state through the activation kinases, Cdk2 and Cdc7. So far, the results obtained in this specific research suggest that Cdk2 kinase activity is not essential for the switching of hypoxic pre-initiation state for the active replication. However, the inhibition of Cdk2 kinase activity fails to affect the phosphorylation and translocation of Cdc6 after reoxygenation. The mechanism of phos-phorylation of Cdc6 triggered by reoxygenation is still unclear, to be determined. Next kinase, Cdc7 is phosphorylated after reoxygenation and thereby Cdc7 phosphorylates its substrate MCM2 after reoxygenation and results in dissociation of MCM2 from chromatin. Thus, Cdc7 kinase may be a possible switch for reoxygenation to release of hypoxic replicon arrest and succeeding synchronous wave of replicon initiation.

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