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Cell cycle checkpoints — molecular background

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Cell cycle checkpoints are the surveillance mechanisms monitoring both the fidelity and accuracy of DNA replication and the segregation of chromosomes. By delaying progression through the cell cycle, checkpoints provide more time for repair before the critical phases of DNA replication and ensure the proper segregation of chromosomes during mitosis. The paper provides basic information about the molecular mechanisms operating in various cell cycle checkpoints activated by DNA damage or disturbances in mitotic spindle assembly.

key word: Cdk kinases, ATM, p53, DNA damage, mitotic spindle

INTRODUCTION

The cell cycle consists of DNA synthesis (S) and mitosis (M) phases separated by gap (G) phases in the order G1-S-G2 M. It is driven by the sequential activation of a number of Ser/Thr protein kinases that become active when they associate with their respective regulatory cyclin subunits. Each of these cyclin-dependent kinases (Cdk kinases) phosphorylates and therefore alters the proteins required for the execution of specific cell cycle events. The activities of Cdk kinases are regulated by changes in the supply of the cyclin subunit (proteins that exhibit the characteristic pattern of appearance and disappearance during the cell cycle), by inhibitory or activatory phosphorylations of the catalytical subunit and by association with different inhibitory proteins (Cdk inhibitors) [1, 5]. Progression from one phase of the cell cycle to the next can be initiated only after passage through what are known as checkpoints (a concept introduced by L. Hartwell, Nobel laureate in 2001 [7]), where correct completion of the preceding steps is verified. Checkpoints, therefore, may be considered as surveillance mechanisms controlling the order and timing of cell cycle events and ensuring that biochemically independent processes are completed. A delay in a critical cell cycle process will cause a delay in all other events of the cell cycle progression. If the damage is irreparable, checkpoint signalling activates pathways that lead to programmed cell death. Loss of checkpoint integrity can allow the propagation of DNA lesions and result in permanent genomic alterations. Most of the information about molecular mechanisms and proteins involved in cell cycle checkpoints has been obtained in genetic studies of budding and fission yeasts, in which mutants defective in particular phases of the cell cycle were produced. Nowadays we have already discovered that an essential mechanism for the maintenance of genomic integrity exhibits a high degree of evolutionary conservation in eukaryotes and that multiple mechanisms could control the same cell cycle transition point. This enables the checkpoint to respond to different types of stress and apply precise control.

Two main classes of checkpoint can be distinguished, one connected with cellular responses to DNA damage and the other occurring during mitosis.

DNA damage-induced checkpoints

Checkpoints activated by various types of DNA damage may occur at the border of G1/S phases (G1 phase checkpoint), during the S phase (intra-S phase checkpoint, DNA replication checkpoint, genome

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integrity checkpoint), during G2 and at the border of G2/M phases (G2 damage checkpoint, G2/M phase checkpoint) [11]. These were originally defined as regulatory pathways that control the ability of cells to arrest the cell cycle in response to DNA damage, allowing time for repair. It is now known that, in addition to controlling cell cycle arrest, these pathways also control DNA repair pathways, various transcriptional programmes and telomeres length as well as induce routes leading to cell death by apoptosis.

Three major groups of proteins: sensors, transducers and effectors are known which act in concert to translate the signal of damaged DNA into a cellular response. As sensor proteins that recognise damaged DNA directly or indirectly and signal the presence of abnormalities the following are proposed: poly(ADP-ribose) polymerase, DNA-dependent protein kinase, as well as proteins related in structure to PCNA (Rad 9, Rad 1, Hus 1) or subunits of replication factor C (Rad 17). Among transducer proteins two protein kinases, ATM and ATR, related to the phosphoinositide 3-kinase, play the central role in the entire DNA damage response [8]. These amplify the damage signal from the sensors by phosphorylating other kinases (e.g. checkpoint kinases Chk1 and Chk2) and other effector proteins involved in DNA repair, transcription regulation and cell cycle control such as BRCA1, Nbs1(Nibrin), p53 and phosphatase Cdc25.

In the regulation of the G1 checkpoint the tumour suppressor protein p53 is the major damage effector protein [2]. It prevents the onset of the S-phase through transcriptional regulation of p21, a Cdk kinases inhibitor, as well as GADD45, a protein able to complex with PCNA, or 14-3-3, proteins that recognise and bind proteins containing phosphorylated serines. As a result, the activity of Cdk2 kinase complexed with cyclin E, necessary for S-phase initiation, is suppressed. In this situation suppressor protein Rb remains unphosphorylated and bound to transcription factor E2. Moreover, the transcription of genes coding proteins required for DNA synthesis is still blocked. An elevated intracellular level of p53 protein is observed in cells treated with ionising and UV irradiation, mainly as a result of its increased stability. Phosphorylation of p53 by ATM on Ser15 and by ATM-activated Chk2 kinase on Ser20 prevents its targeted degradation in proteasome 26S. Additionally, phosphorylation by ATM of MDM2 cellular protein, acting as ubiquitin ligase, interferes with its ability to bind p53, which contributes to the stabilisation and nuclear accumulation of p53.

The S-phase checkpoint is of particular importance, since duplicaton of the genome takes place in the S phase and repair of damaged DNA in this phase may be the final line of defence to eliminate DNA lesions before they are converted into inheritable mutations. A number of mechanisms have been identified for the attenuation of the S-phase in response to DNA damage. The main mechanism includes sequential action of ATM and Chk2 kinases, which leads to inhibitory phosphorylation of Cdc25A phosphatase, responsible for Tyr15 dephosphorylation and therefore activation of all Cdk kinases. The other is the pathway ATM-Nbs1, which probably participates in the inactivation and subsequent degradation of Cdc25C phosphatase. The other downstream factors of this pathway, probably proteins involved in DNA replication, are yet to be identified.

In the G2 phase checkpoint, which controls the transition from the G2 phase to mitosis, the phosphatase activity of Cdc25C is of special importance, as it activates Cdk1 kinase complexed with cyclin B or A, indispensable for mitosis. Phosphorylation of Cdc25C phosphatase by Chk1 kinase, activated either by ATM or ATR, provides an effective G2/M block upon recognition of DNA damage.

Checkpoints during mitosis

During mitosis at least two checkpoints are present. The first is named the CHFR checkpoint, after a gene encoding a protein which possesses Forkhead-associated and RING finger domains as well as ubiquitin ligase activity [3]. This occurs during prophase when CHFR protein inhibits chromatin condensation and therefore entry into the metaphase. The second checkpoint is situated between the metaphase and anaphase and is named the "spindle assembly" checkpoint, mitotic checkpoint, or spindle checkpoint [10]. It monitors the microtubule structure and chromosome attachments to the mitotic spindle and delays chromosome aggregation during the anaphase, until defects in the mitotic spindle apparatus are corrected. Crucial constituents of this checkpoint are the kinetochore-associated proteins, designated as MAD2, BUBR1, BUB1 and BUB2, which are able to form multicomponent complexes. These act in concert and regulate (especially MAD2 and BUBR1) mitotic progression by direct in-

teraction and inhibition of the APC ubiquitin ligase responsible for the proteolytic degradation of multiple protein substrates, such as cyclins B and A and securin Pds1 [4]. Their proteolysis at the metaphase/ /anaphase transition, as well as disruption of the microtubules, allows sister chromatid separation and further progression of the cell cycle.

When checkpoints fail…

The question as to whether a causal relationship exists between checkpoint failure, genomic instability and cancer is of special importance. It is already well documented that genetic alterations in genes encoding signalling molecules that act in the various cell cycle checkpoints may lead to tumourigenesis. Mutations in the p53 tumour suppressor gene, common in the majority of human cancers, cause the inability of mutated p53 protein to precisely control the expression of genes coding some constitutive cell cycle machinery components as well as proapoptotic genes. Moreover, mutated p53 protein cannot be phosphorylated by kinase Chk2. Inactivating mutations of ATM, BRCA1/2 and Nbs1 genes cause defects in DNA damage signalling, give rise to some forms of chromosomal instability and increase the risk of cancer, especially of leukaemia and lymphoma. The presence of mutated mitotic checkpoint genes such as CHFR, BUBR1 and MDM2 in various tumour cells has also been reported. It is obvious that understanding of the molecular mechanisms of cell cycle regulation and checkpoint abnormalities in tumours offers an insight into potential therapeutic strategies [6, 9, 12].

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