

Cancer and the Cell Cycle

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Abstract The purpose of this short review is to provide an overview of mammalian somatic cell cycle events and their controls. Cell cycle-related studies have been under way for only 5% of this millennium, yet since then nearly 20,000 references have appeared. This vast literature cannot be detailed here, nor can fundamental information obtained with other organisms such as yeast and *Xenopus*, or topics such as the abbreviated cell cycle in early embryonic cells. (General references include Murray and Hunt [1993] *The cell cycle, an introduction*. New York: Oxford University Press, and Denhardt [1999] In: *The molecular basis of cell cycle and growth control*. p 225–304. New York: John Wiley & Sons, Inc.) *J. Cell Biochem. Suppl.* 32/33:166–172, 1999. © 1999 Wiley-Liss, Inc.

Key words: cell cycle; cancer; literature

THE BASIC SOMATIC CELL CYCLE

History

Before the twentieth century, mitotic and interphase cells were revealed by microscopy. In mid-century, the cell cycle phases G1 (Gap1), S (DNA synthesis), G2 (Gap 2), and M (mitosis) were defined by Swift and by Howard and Pelc [1953]. Subsequent pioneering work on cell physiology refined the cycle, which has a duration between cell divisions of about 1 day (G1 8 h, S 8 h, G2 2 h, M 1 h). Important early technical innovations included a variety of cell synchronization methods to investigate cycle progression.

This research gave rise in 1971 to the discovery of cell cycle regulation by Masui and Markert, who found an activity in *Xenopus* oocytes, termed maturation promoting factor (MPF), that could promote meiotic cell cycle progression. The idea of cell cycle regulation was further promoted in 1974 by Hartwell and his colleagues with genetic studies that revealed 73 cell division cycle (cdc) mutants [Hartwell and Weinert, 1989] and in mammalian cells by Pardee [1989] with studies on

deranged G1 growth control of cancer cells. Biochemistry and molecular biology soon followed with the identification of cyclins in 1983. These proteins were found by Hunt, Ruderman, and colleagues to vary in abundance throughout the cell cycle because of changes in their synthesis, phosphorylation, and destruction [Minshull et al., 1989]. The functional identification of the cyclin dependent kinases (cdks) by Nurse [Norbury and Nurse, 1992] soon followed, and it was shown that cdks are activated by cyclins to phosphorylate specific substrates at certain points in the cell cycle, an activity necessary for cell cycle progression. Historical summaries include those by Baserga [1985] and Cross et al. [1989].

The G0 State

In vivo, most mammalian cells are quiescent; few are cycling at any time. In culture, noncancer cells, unlike cancer cells, can be made quiescent (in G0) by cell-cell contacts at high density or by serum or nutrient deprivation. They require anchorage to a solid surface for growth, and in suspension are arrested in G0. Quiescent cells generally have unduplicated DNA, but otherwise differ from G1 cells, which express many biochemical proliferation markers such as Ki-67.

G1 Phase

Cells in G0 are activated to proliferate by growth factors, such as epidermal growth factor

Grant sponsor: National Institutes of Health; Grant number: CAR01-CA61253; Grant sponsor: Susan G. Komen Foundation; Grant number: 9862; Grant sponsor: NRSA; Grant number: 1F32-CA79197-01.

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Received 23 September 1999; Accepted 30 September 1999

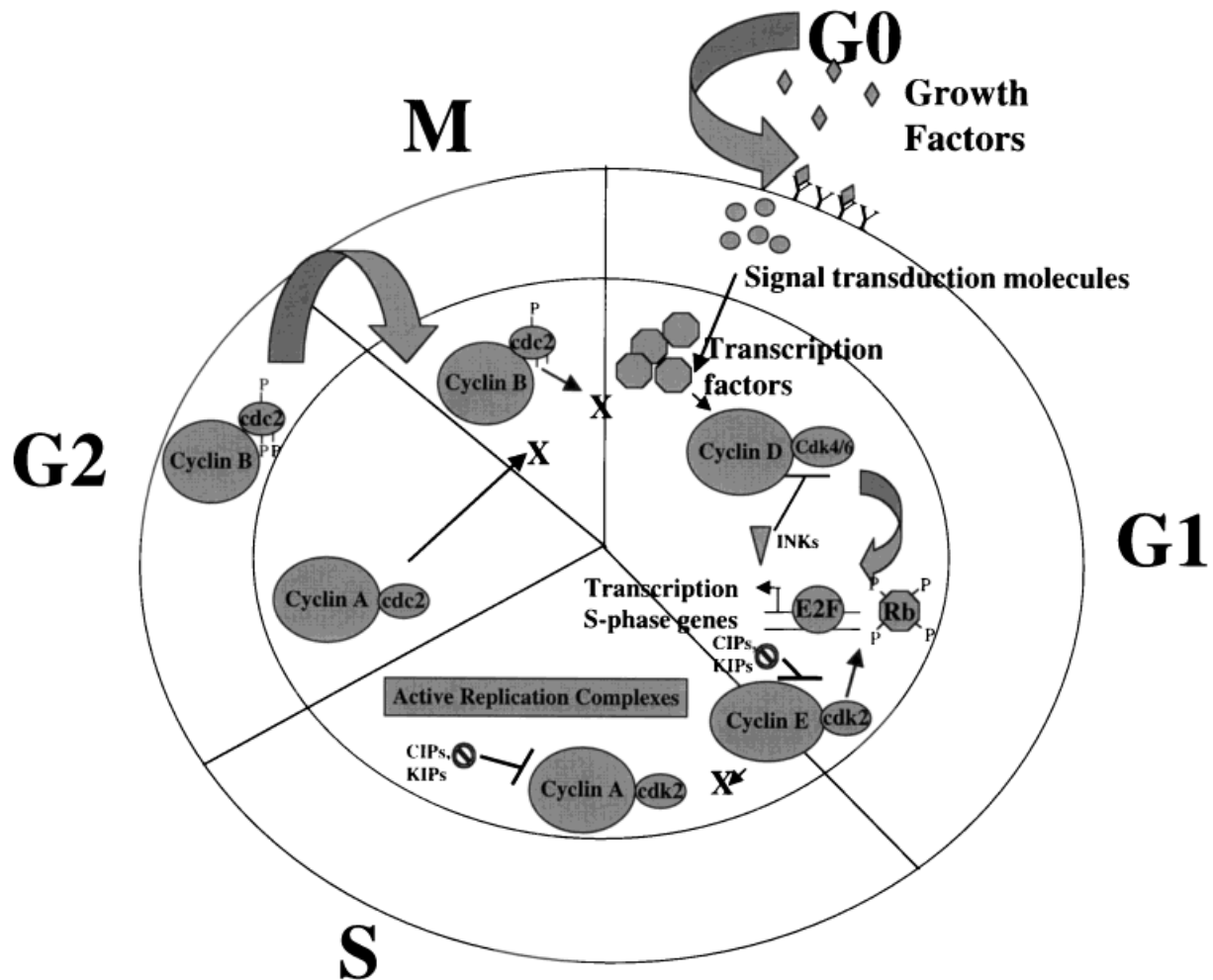


Fig. 1. Schematic view showing the four phases of the cell cycle and some of the many molecules important for progression. Extracellular events such as growth factor stimulation induce signal transduction cascades that begin in the cytoplasm (outer circle) and end in the nucleus (inner circle), where transcription factors are activated, leading to the synthesis of cyclin D and subsequent activation of the cyclin D/cdk 4/6 complexes. The cyclin D/cdk complex then phosphorylates downstream targets, including the Rb protein, which releases repression of E2F and permits transcription of S-phase genes. In

addition, cyclin D/cdk activity leads to activation of cyclin E/cdk2 complexes and DNA synthesis. This propels the cell through the remainder of the cell cycle, with cyclin A playing a role in S-phase progression and cyclins A and B acting in mitosis. The cyclins are regulated by a variety of factors, including the CKIs (INKs and CIPs/KIPs), phosphorylation, and subcellular localization, only some of which are represented. Progression through the cell cycle is also regulated by cyclin degradation. X, degradation of the respective cyclin.

(EGF) and insulin-like growth factor (IGF), advancing to the G1 phase of the cell cycle several hours after stimulation (Fig. 1). If the growth factors are removed early in G1, cells will revert to a quiescent state. Growth factors act by binding extracellularly to their specific transmembrane receptor proteins. This binding activates the receptor's intracellular kinase, initiating a multistep signal transduction kinase cascade that involves the products of many genes including, for example, ras, fos, myc, and the MAP and PI-3 kinases. In particular, the ras proto-oncogene is important for cells to exit the quiescent state and to pass through the

G1/S transition [reviewed by Kerkhoff and Rapp, 1998].

This signal transduction cascade leads to the activation of G1, which results in the expression of at least 100 genes [Pestov and Lau, 1994]. Central is cyclin D, which, as a result of growth factor stimulation and ras induction, increases in early-mid G1, thereby activating the cyclin-dependent kinases cdk4 and cdk6 [Sherr, 1995]. Active cyclin D/cdk complexes phosphorylate the retinoblastoma protein (pRb). Rb is a tumor-suppressor protein, which is a component of the Rb/E2F transcription complex.

Underphosphorylated Rb was recently shown to recruit HDAC1 histone deacetylase to the complex, which modifies chromatin structure and causes transcriptional repression [Magnaighi et al., 1999]. When Rb is phosphorylated, it is released from the E2F complex, enabling the transcription of numerous genes involved in DNA synthesis. The primary cell cycle target of the cyclin D/cdk cascade is cyclin E, which activates cdk2 and DNA synthesis [Geng et al., 1999].

In addition to the *de novo* synthesis of cyclins in G1, which activate cdk function, phosphorylation of the kinases provide another level of regulation. For instance, CAKs are cdk activating kinases necessary for cell cycle progression that phosphorylate the threonines of the T loop of cdk, thus exposing the catalytic cleft and enabling substrate binding. Differential phosphorylation of the cdk is found at both the G1/S and G2/M transitions of the cell cycle. A third level of regulation involves compartmentalization of cyclin/cdks within the cell [reviewed by Morgan, 1995]. These regulatory mechanisms will be further discussed below.

Whereas cyclins activate cdks, inhibitors of the cdks (CKI) have been identified that can block G1/S progression by binding to cyclin/cdk complexes. The CKIs thus balance with cyclins the activity of cdks in cycling, development and tumorigenesis [Sherr and Roberts, 1999]. These inhibitors belong to two families: The INK4 family, comprised of p15, p16, p18, and p19, which act primarily on cyclin D/cdk4/6; and the CIP/KIP family comprised of p21, p27, and p57, which are less specific and can inhibit several cyclin/cdk complexes. Increased levels of cyclin D and E throughout G1 allow cells to overcome the inhibition by CKIs of cdk activity, initiating the events necessary for progression into S-phase.

Remarkably, cells cannot pass beyond a specific point in G1, named the Restriction Point, or R-point, in mammalian cells [Pardee, 1974] and START in yeast [Hartwell et al., 1974], if growth factors or nutrients are insufficient. They will then instead enter quiescence (G0). However, once beyond this point the cells are committed to divide and no longer require extracellular growth factors to complete the cycle. The final step that is needed to pass this restriction point in late G1, where growth factor stimulation is no longer necessary, requires synthesis of an unstable protein that is most likely cyclin E. This protein is a strong candidate because it

appears about the same time as the R point, and is overproduced in tumor cells. In Rb defective cells, cyclin D1/cdk4 is dispensable for the G1/S transition, whereas cyclin E/cdk2 is not. Furthermore, Geng et al. [1999] recently demonstrated that cyclin E could rescue the defects in a cyclin D1-deficient mouse, even in the absence of Rb phosphorylation. These data suggest that cyclin E is very likely the R-point protein.

S Phase

DNA replication, the mark of S phase, originates on the nuclear matrix at about 50,000 origins of replication, approximately 100 kb apart. Different genes are duplicated from origins of replication in mammalian cells at specific times in S phase, with transcriptionally active genes in euchromatin replicating early in S-phase, and transcriptionally inactive genes in heterochromatin replicating late [Goldman et al., 1984]. Recent work, particularly in yeast, has identified several of the proteins important for initiation of DNA replication [for review, see Stillman, 1996; Toone et al., 1997].

Early in S phase, cyclins D and E are targeted by ubiquitination to be degraded by proteasomes [King et al., 1996; Elledge and Harper, 1998]. At this time, cyclin A levels rise, activating cdk2 and enabling S-phase progression. In addition, enzymes as well as other proteins involved in DNA synthesis increase in amount at the beginning of S phase, but they are not rate-limiting. These include histones, proliferating cell nuclear antigen (PCNA), thymidylate synthase, and ribonucleotide reductase. The transcription of thymidine kinase is regulated at the G1/S boundary by a promoter complex that contains an Rb-like protein p107, cyclin A, and cdk2 [Li et al., 1993], providing another link between cell cycle proteins and transcriptional activation of S-phase genes. Furthermore, these proteins move from the cytoplasm into the nucleus at the G1/S boundary, a process controlled by an unknown mechanism. These regulatory acts all aid in the progression of S-phase. (For review of the S-phase, see Dalton [1998] and Ford and Pardee [1998].)

G2 Phase

Cells prepare for mitosis during late S and throughout G2, in part by increasing the levels of cyclins A and B. Cyclin B is believed to be the main mitotic cyclin, but cyclin A, although

mostly involved in S-phase events, is also essential for cells to enter mitosis.

As cyclin B levels rise, it forms a complex with *cdc2* (*cdk1*). This primes *cdc2* for an activating phosphorylation at Thr161 by CAK. However, the complex is still held inactive in G2 by inhibitory phosphorylation at Thr14 and Tyr15, both in the active site of the kinase. The enzymes that regulate phosphorylation and dephosphorylation of Thr14 and Tyr15 are the *wee1* kinase and the *cdc25* phosphatase. During G2, *wee1* kinase activity is greater than *cdc25* phosphatase activity, thus keeping cyclin B/*cdc2* inactive. Cyclin B/*cdc2* activity is further regulated by its subcellular localization. The complex formed in G2 remains in the cytoplasm until mitosis, when it is finally shuttled to the nucleus [reviewed by Jackman and Pines, 1995].

M Phase and Cytokinesis

Mitosis is dependent on the completion of S phase, although drugs can make it independent. Proteolysis of the *wee1* kinase in a *cdc34*-dependent fashion is required for entrance into mitosis and is inhibited if DNA replication is blocked [Michael and Newport, 1998]. In addition to *wee1* proteolysis, *cdc25* is activated at prophase by a regulatory phosphorylation, which in turn leads to cyclin B/*cdc2* activation. At approximately the same time, cyclin B/*cdc2* is rapidly relocated to the nucleus. Thus, cyclin B/*cdc2* activity is tightly controlled by both location and phosphorylation [reviewed by Jackman and Pines, 1997].

Once cyclin B/*cdc2* is fully activated, a shift in the balance of kinase to phosphatase activities leads to large increases in the phosphoprotein content of the cell, believed to be important for the dramatic morphological changes accompanied with mitosis. These changes include nuclear envelope breakdown, disassembly of the microtubule network and rearrangement into mitotic spindles, reorganization of the cytoskeleton, and chromosome condensation. These events prepare the cell for its division. [For a review of cytokinesis, see Field et al. [1999].]

Mitotic progression, as well as exiting mitosis, depends on the anaphase-promoting complex (APC)/cyclosome, which functions in ubiquitin-mediated proteolytic events. The APC/cyclosome recognizes a conserved 9-residue motif called the destruction box in mitotic targets, including cyclins A and B. By a mechanism not yet clearly understood, the mitotic

cyclins are degraded in a defined order, with the degradation of cyclin A preceding that of cyclin B. Cyclin B degradation enables the cells to exit mitosis [Koepp et al., 1999].

The Next Cycle: Licensing for DNA Synthesis

DNA synthesis is not reinitiated until after mitosis is completed and the mitotic cyclins are degraded. The process that permits only one DNA replication per cycle is named licensing [Chong et al., 1996]. Involved in DNA licensing are MCM proteins, ORC proteins (which bind to the origins of replication), and CDKs. One model suggests that, after mitosis, a fall in CDK activity allows MCM proteins to bind the ORCs, forming a prereplicative complex, which includes *Cdc6/cdc18* proteins. As CDK activity rises at the end of G1, DNA replication is activated. After activation of the replicative complex, *Cdc6/cdc18* proteins are degraded, irreversibly inactivating the complex. Not until the end of mitosis, when CDK activity declines, are the MCM proteins returned to a dephosphorylated state, allowing them to reinitiate binding to the ORCs and to restart the cycle [Wuarin and Nurse, 1996].

Drugs including staurosporine break down the dependence of DNA synthesis on a prior M phase, particularly if the cells are p21 negative. When induced by anticancer agents, cells that have lost p21 undergo multiple rounds of DNA synthesis without mitosis [Waldman et al., 1996].

MODIFICATIONS OF THE CELL CYCLE

Checkpoints

Aside from the normal cell cycle, regulatory processes exist such as the restriction point [Pardee, 1974] or START, and the blocks resulting from DNA damage [Busse et al., 1977]. These control events were named checkpoints [Hartwell and Weinert, 1989] and have been reviewed frequently [Elledge, 1996; O'Connor, 1997]. The R point is regulated by the INK4 proteins (p15, p16, p18, p19), which block cyclin D-dependent kinase activity, thereby preventing phosphorylation and inactivation of Rb [Sherr, 1996]. This in turn prevents the synthesis and activity of cyclin E, without which the cells cannot progress through the R point.

DNA Damage Checkpoints

Deoxynucleotide depletion, or as few as one double-strand break in DNA, can create a ma-

major checkpoint. The checkpoints are necessary to prevent mutations by halting the cell cycle and increasing the time for DNA repair to occur before entry into S-phase or mitosis.

DNA damage activates ATM (rad3), the gene that causes high sensitivity to x-rays and high tumor incidence when mutated in ataxia-telangiectasia. ATM phosphorylates the p53 protein in response to DNA damage [Banin et al., 1998; Canman et al., 1998]. This phosphorylation is important for the biological activity of p53, leading to the activation of p21. p21 blocks cyclin/cdk activities, inducing a G1 arrest. Furthermore, p21 may play a role in DNA repair through binding to PCNA. In addition to p21, p53 induces Gadd45, another protein involved in DNA repair.

DNA damage creates a second checkpoint at G2/M. Both the G1/S and G2/M checkpoints are affected by similar factors, including p53, GADD45, and by various drugs. Although p53 can induce a G2 delay, its function is not required for the G2/M checkpoint, as p53 defective cells are still capable of arrest. Instead, p53 is required for sustaining the G2 arrest [Bunz et al., 1998]. Thus, cells that are lacking p53 may be more susceptible to genetic instability because of its importance in both the G1/S and G2/M DNA damage-induced arrests.

Central to DNA damage-induced G2/M arrest is the activation of the chk1 kinase, which causes an inhibitory phosphorylation on the cdc25 phosphatase [Enoch et al., 1998]. This phosphorylation allows cdc25 to bind to the 14-3-3 protein, which holds the phosphatase in the cytoplasm and prevents the removal of the inhibitory phosphorylation on Thr14 and Tyr15 of cdc2, thereby maintaining cyclinB/cdc2 in an inactive state [Yang et al., 1999].

Other molecules typically not thought of as cell cycle regulators have also been implicated in DNA-damage induced G2/M arrest. These include several homeobox genes, transcription factors involved in development, such as HOX11 and HSIX1. When overexpressed, both HOX11 and HSIX1 can attenuate the DNA damage-induced G2/M cell cycle checkpoint [for review, see Ford, 1998]. This may provide a mechanism by which HOX11 and HSIX1 play a role in cancer.

DNA damage initially produces a checkpoint arrest that provides time for repair. But if repair is not soon completed, the cells retain the mutations or undergo apoptosis. G1 checkpoint genes, including p53, are also involved in apop-

tosis [Reed, 1999]. Wild-type p53 activates the BAX gene, which inhibits the ability of bcl2 to protect cells against apoptosis. In addition, bcl2 mRNA levels decline after p53 activation. Therefore, p53-dependent apoptosis may be caused by an increase in the ratio of BAX to bcl2 [reviewed by O'Connor, 1997]. Different cells show various apoptotic responses. It remains unclear what makes one cell type become arrested in response to DNA damage, while another may apoptose.

M Phase Checkpoints

During mitosis, accurate chromosome segregation is dependent on attachment to and alignment on the mitotic spindle before activation of the cyclin proteolysis machinery that induces sister chromatid separation. To ensure that this process occurs correctly, the spindle assembly checkpoint delays anaphase until all chromosomes are attached to the mitotic spindle. Proteins involved in this checkpoint include members of the MAD and BUB families, first identified in budding yeast. These proteins bind to unattached kinetochores and prevent progression through mitosis by inhibiting APC-mediated proteolysis of anaphase inhibitors [Nasmyth, 1999].

Cell Aging

The normal cell cycle outlined above is modified by various factors. One of these is cell age. Human cells cease growing after about 50 cycles, as shown by Hayflick. It is believed that telomere length, which decreases upon each cell cycle, may provide a "clock" for aging. Furthermore, the p21 CKI was discovered in aging cells, and increases before the final arrest in G1 phase [for review, see Wynford-Thomas, 1997].

Cancer

The hallmark of cancer is deranged growth control [Pardee et al., 1978]. Checkpoints are defective in cancer cells [Hartwell and Kastan, 1994]. Control mechanisms are usually lost by mutation, for example many cancers lose or have mutated p53 genes, or have alterations in some components of the Rb pathway. In addition, carcinogenic viruses such as SV40 produce proteins, such as T-antigen, which bypass G1/S control and transform cells, mainly by eliminating p53 and pRb [reviewed by Sherr, 1996].

Defective checkpoint mechanisms in cancers, as well as defective DNA repair, produce fur-

ther chromosomal aberrations and genomic instability [Nojima, 1997]. Thus checkpoint controls limit the appearance of genetic variants and the progression of cancer. Caffeine or 2-aminopurine removes the G2/M block; as a consequence most cells die. However, some cells will survive such treatments, and have increased chromosomal abnormalities. These results demonstrate the protective role of the G2/M checkpoint against damage-induced chromosomal mutations [Fingert et al., 1986].

Hereditary defects in checkpoint control are well known to be carcinogenic, presumably due to the increased rates of mutation incurred when cells continue to cycle without repairing DNA damage. For example, p53 abnormalities, which cause checkpoint defects, are correlated with an increased frequency of deletion of the breast cancer locus BRCA1 and an increased rate of breast cancer [Tseng et al., 1997]. Ataxia-telangiectasia, Nijmegen breakage syndrome, Li-Fraumeni syndrome, Fanconi anemia, and Bloom's syndrome are all associated with defects in cell cycle checkpoints and cancer susceptibility.

Defective cell cycle regulation and checkpoint mechanisms have implications for cancer detection and treatment. For example, because both cyclin D and cyclin E mRNAs are elevated in many cancers, they could be followed for diagnostic purposes. In addition, since the goal of therapy is to selectively kill cancer cells, which often have defective checkpoint mechanisms, these cells may be more susceptible to DNA damaging agents than are their normal counterparts. Creation of defective checkpoint mechanisms in cancer cells can be applied to produce selective lethality of DNA damaging agents, in G1/S [Pardee and James, 1975], and in G2/M by pentoxifylline [Fingert et al., 1986].

In addition to cell cycle checkpoints, cyclins and cdks provide novel targets for cancer treatment. Chemical inhibitors of cyclin-dependent kinases include Olomoucine [Vesely et al., 1994]. Recently, a novel peptide was synthesized that serves as a docking site for cyclin/cdk2 complexes, inhibiting their action and inducing apoptosis preferentially in tumor cells [Chen et al., 1999].

Clashes of conflicting control signals can cause apoptotic death [Evan, 1992]. Therapy with drugs that create multiple cell cycle arrests by engaging both the G1/S and the G2/M checkpoints are promising, as seen with a com-

bination of β -lapachone and taxol [Li et al., 1999].

CONCLUSION

Mechanisms controlling growth of normal and diseased cells, as in cancers, have been described in considerable detail during recent decades. There is, of course, much still to learn about the molecules involved, transcriptional controls, regulatory phosphorylation, RNA and protein turnover, and molecular compartmentalization that influences the cell cycle. These current and future findings will provide novel approaches to understanding and treating major diseases.

ACKNOWLEDGMENTS

This work was aided by grants from the National Institutes of Health, grant CA01-CA61253; from the Susan G. Komen Foundation, grant 9862; and by an NRSA Fellowship (to H.L.F.), 1F32-CA79197-01.

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