Signal Transduction of Cell-Cycle Regulation: Its Temporo-Spacial Architecture

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Cell growth and proliferation are the most fundamental functions of life. Ironically, however, such functions underlie the two principal causes of death in humans living in developed countries today i.e., neoplasms and proliferative vascular disorders. Despite the significance in both science and medicine, studies on the molecular signalling of cell cycle progression have been hampered by both spacial and temporal complexities. It takes at least several hours for mitogen-stimulated cells to traverse through the first gap (G1) phase before entering the DNA synthetic (S) phase when fulfilling a complete series of signal transduction from cell surface receptors to DNA replication machinery. Major breakthroughs have been achieved during the last decade: First, the investigation of viral oncopgenes has led to the identification of crucial cellular molecules involved in mitogenic signal transduction; and second, the basic structure of cell cycle machinery emerged as the result of the historic convergence of two independent lines of investigation, one in oocytes and the other in yeasts.

Discoveries of Mitogenic Signalling Molecules: Lessons from Viral Oncogenes

In 1910, Rous showed that the cell-free filtrate of chicken sarcoma (Rous sarcoma) was capable of inducing new sarcomas in injected chickens. His observations were not well appreciated those days, but decades later, in 1970, the causative agent Rous sarcoma virus was isolated [1]. Since then, numerous tumor-inducing retroviruses have been isolated from avian as well as mammalian tumors, and the oncogenes responsible for inducing tumors in host animals have been identified [2, 3]. Those studies revealed that the viral oncogenes of tumor-forming retroviruses encode mutated, constitutively active counterparts of cellular proto-oncogene products, which turned out to be a wide variety of signalling molecules that are indispensable for host cell growth and/or proliferation under physiological conditions [4–6]. These include growth factors, receptors, GTP-binding transducer proteins, protein kinases and nuclear transcription factors (Fig. 1a). For example, v-src, the viral oncogene of the Rous sarcoma virus, encodes the oncoprotein pp60<sup>src</sup> which, by virtue of the lack of an inhibitory phosphorylation site, is a constitutively active form of the cellular proto-oncogene product pp60<sup>src</sup>, a 60 kDa phosphoprotein with membrane-bound non-receptor type protein tyrosine kinase activity. In addition, the oncogenes of DNA tumor viruses are designed in such a way that their products should specifically bind to and inhibit cellular tumor suppressors [7] (Fig. 1b). Among others, pRb and p53 are the two most well characterized tumor suppressors which, in normal cells, play critical roles in cell cycle checkpoint control [8–10]. Deletion and loss-of-function mutations in these genes are associated with both inherited and sporadic human malignancies. Especially, mutations of p53 constitute one of the most common genetic changes found in sporadic forms of human neoplasms. Germ line p53 mutations cause a familial syndrome of multiple neoplasms (Li-Fraumeni syndrome). p53 turned out to be a transcription factor responsible for the induction of an inhibitor for cyclin-dependent kinases in response to genotoxic stress such as ultraviolet irradiation (see below).

Another frequent target of mutation found in
Mitogenic Stimuli Turn on the Ras Signalling System

Molecular mechanisms for Ras activation in response to external stimuli

Ras is a 21 kDa GTP-binding protein located at the inner surface of the plasma membrane, and has been found to be conserved in every eukaryote examined thus far. A recent investigation provided evidence that Ras functions as a molecular switch for activation of multiple mitogenic signalling pathways. When serum-deprived, quiescent cells are stimulated with such diverse mitogens as growth factors, G protein-coupled receptor (GPCR) agonists and hemopoietic cytokines, cellular Ras is activated rapidly (within a minute) by the replacement of bound GDP by GTP [13–16]. The GDP/GTP exchange in Ras is catalyzed by Ras-guanine nucleotide exchange factors (Ras-GEFs) [11, 12]. mSOS, the mouse homologue of Drosophila son of sevenless (dSOS), which is involved in Ras-dependent eye development in Drosophila, is one of the most well characterized Ras-GEFs (Fig. 2). In quiescent cells, mSOS exists in a cytosolic complex with an adaptor protein, growth receptor binding protein 2 (GRB2)/Ash. GRB2 is composed of three domains: two src homology-3 (SH3) regions flanking one SH2 region; and the SH3 regions of GRB2 are tightly bound to a proline-rich region of mSOS. Growth factor stimulation of the receptor/protein tyrosine kinases results in rapid trans-autophosphorylation and dimerization of the receptor molecules, as well as tyrosyl phosphorylation of other substrate proteins such as insulin receptor substrate-1 (IRS-1) and SHC. Specific phosphotyrosine-containing amino-acid sequences on the cytoplasmic domains of the receptors or on the substrate proteins serve as docking sites for SH2 regions in GRB2. The binding of GRB2 to these docking sites results in the recruitment of the mSOS/GRB2 complex to the plasma membrane, allowing interaction of mSOS with Ras [17, 18]. In addition to growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and insulin, agonists for seven-transmembrane GPCRs induce mitogenesis in a wide variety of cells. These include thrombin, lysophosphatidic acid (LPA), bombesin, endothelin, serotonin and muscarinic receptor agonists [19–25]. It has been demonstrated that GPCR agonists activate Ras via the βγ [26] or the α [27] subunits of heterotrimeric GTP-binding proteins through mechanisms involving src family non-receptor type tyrosine kinases such as Lyn, Syk and Fyn [28, 29], and the adaptor protein SHC [28, 30, 31].

In neuronal tissues where Ras plays a role in medi-
Fig. 2. The Ras signalling system. The mechanisms for growth factor–induced Ras activation and Ras-mediated activation of downstream effectors are summarized. GF, growth factor; GFR/PTK, growth factor receptor with protein tyrosine kinase activity; R-subst., cellular substrate for GFR/PTK; FKBP12, FK506 binding protein 12; FRAP, FK 506 binding protein-rapamycin–associated protein; S6, ribosomal 40S subunit S6 protein. See text for details.

ating signals from neurotransmitters to gene expression, a brain specific Ras-GEF termed Ras-GRF is utilized for Ras activation [32, 33]. It has been reported for neonatal rat brain explant that muscarinic receptor activation leads to an increased Ser and/or Thr phosphorylation and the activity of Ras-GRF [32] through a mechanism involving the \( G_{\text{py}} \) subunit. It was also demonstrated that when cortical neurons are depolarized by high KCl solution, Ca\(^{2+}\) influx activates Ras by increasing Ras-GRF activity through the direct binding of Ca\(^{2+}\) and calmodulin (CaM) to Ras-GRF [33]. In addition, the involvement of a protein tyrosine kinase (Pyk2) is implicated in Ras activation [34]. Pyk2 becomes activated in response to the elevation of [Ca\(^{2+}\)], or activation of protein kinase C (PKC) [34]. Thus, a variety of stimuli that activate the Ca\(^{2+}\) messenger system may lead to Ras activation via Pyk2-mediated phosphorylation events.

The Ras effector systems

Active Ras in its GTP-bound form takes a conformation that is capable of interacting with a number of downstream effector molecules through a region called the “effector loop” localized at residues 32–40 [35]. Direct Ras effectors thus far identified include the Raf family of serine/threonine protein kinases [36], a lipid kinase phosphatidylinositol-3-kinase (PI3K) [37], Raf-GEF, which is the guanine nucleotide exchange factor for the Ras family protein Ral [38], Ral-GEF-like molecules (RGLs) and the \( \zeta \) isoform of protein kinase C (PKC\( \zeta \)) [39]. In addition, the rho family of low molecular weight G proteins including Rac and Rho are likely localized downstream of Ras, at least in certain situations (see below), although the molecular link between Ras and the rho members remains to be elucidated. Ras-GTPase activating proteins (Ras-GAPs), which stimulate the GTPase activity intrinsic to the Ras protein, also interact with Ras at the effector loop. However, several lines of evidence indicate that Ras-GAPs are not likely to act as the effectors of Ras. The inhibition of Ras-GAP activity is reported to underlie PKC-mediated Ras activation in T lymphocytes [40], while Yav, a hematopoietic cell-specific protein with Ras-GRF activity, is involved in Ras activation in response to T-cell receptor activation [41].

The protein kinase cascade consisting of c-Raf1, MEK and MAPK/ERK is one of the most well char-
acterized Ras effector systems [42] (Fig. 2). Thus, upon growth-factor stimulation of quiescent cells, the cytosolic protein kinase Raf1 directly binds through its N-terminal region to the GTP-bound active form of Ras [36], thereby being recruited to the plasma membrane. Further steps including phosphorylation by other kinases (such as src family kinases and ceramide-activated protein kinases) are required for maximal activation of Raf1, which is now capable of activating its substrate, MAPK/ERK kinase (MEK). MEK [43], a dual specificity kinase, in turn activates its sole known substrate, MAPK (also called ERK), by phosphorylating it on essential Thr and Tyr residues [44]. Besides Raf1 and other members of Raf family kinases, there exist at least four MEK kinases that phosphorylate and activate MEK, including Mos [45], MEKK species which are mammalian 98 and 82 kDa kinases [46], and an insulin-activated 56 kDa kinase called MAPKKK [47]. It is also known that, under certain circumstances, the MAPK/ERK pathway is activated in a Ras-independent manner.

MAPK was first identified as a protein kinase activity toward brain microtubule-associated protein-2 (MAP-2), which was undetectable in quiescent cells and stimulated in response to diverse mitogenic stimuli [48, 49]. Therefore, MAPK also stands for mitogen-activated protein kinase. Two classical MAPKs (44 and 42 kDa) are ubiquitously expressed in various tissues and cells. More recently, a group of MAPK-related protein kinases called Jun N-terminal kinase (JNK) or stress-activated protein kinase (SAPK) has been identified, which phosphorylates and activates the transcription factor c-Jun in response to a variety of stress signals [50, 51]. Since that time, the original MAPK have been more preferentially referred to as extracellular signal–regulated protein kinases (ERKS), and the term MAPK has come to be used as the “family name.” Thus, MAPK family kinases include both ERK and JNK/SAPK members. A distinct protein kinase cascade leading to the activation of JNK/SAPK has been identified [52]. ERK and JNK/SAPK kinase cascades are both evolutionarily conserved from yeasts to humans [53], implicating biological significance.

Upon activation, ERKs (p44ERK1 and p42ERK2) translocate to the nucleus within 20 min [54], where they stay for at least a few hours and regulate gene expression by phosphorylating transcription factors. p62(2)TF (also referred to as Elk-1), which is an ets proto-oncogene family transcription factor, is a well-characterized, physiological substrate for ERKs [42]. Thus, ERK-mediated phosphorylation of the ternary complex factor (p62(2)TF) potentiates ternary complex formation together with the serum response factor p67SRF at the c-fos promoter serum response element (SRE) [55], thereby stimulating its transactivation potential. The c-Myc proto-oncogene product is another candidate substrate for ERKs [42]. Other transcription factors such as c-Jun, NF-IL6 and ATF-2 used to be in the list of candidate ERK substrates, however, they are now considered to be physiological substrates of the JNK/SAPK family kinases [50, 51, 56]. ERKs also regulate gene expression via phosphorylation and activation of the cytosolic enzyme p90RSK, which also translocates to the nucleus, phosphorylates the transcription factor Ca(2+)/cyclic AMP response element binding protein (CREB) and increases its transactivation potential, contributing to c-fos induction by mitogenic stimuli [57]. Besides transcription factors, cytosolic phospholipase A2 (cPLA2) is an established physiological substrate for ERKs [58]. A variety of growth factors and GPCR agonists stimulate cPLA2, leading to the release of arachidonic acid and the production of its cyclo-oxygenase metabolite prostanooids, which are considered to be important secondary signaling molecules acting via autocrine/paracrine mechanisms. Agonist-induced activation of cPLA2 depends on both ERK-mediated phosphorylation [58] and recruitment to the plasma membrane by a Ca(2+)-stimulated mechanism [59]; the latter depends on Ca(2+) mobilization from both intra- and extracellular pools [60]. ERKs are also implicated in cytoskeletal organization which takes place at the G2/M border [61].

Studies using dominant negative forms of MEK1 and ERKs or the antisense inhibition of ERK expression demonstrate that activation of the MEK-ERK pathway is essential for mitogen-stimulated quiescent cells to progress into the S phase [62–64]. The enforced expression of a constitutively active form of MEK1 induces DNA synthesis in the absence of external growth stimulation and even malignant transformation [63, 65]. However, activation of the MEK-ERK pathway is not likely sufficient for mitogen-induced G1 to S phase progression under physiological conditions [66–70]. Certain mitogens including insulin [66] and activin [71] do stimulate DNA synthesis without activating the MEK-ERK pathway, at least in some cell types.

In contrast to ERKs, JNK/SAPKs are activated by a number of external stress signals including ultraviolet irradiation, osmotic shock and chemicals such as antibiotics, and link the stress signals to nuclear events. Our laboratory has recently found that mechanical strain on vascular smooth muscle cells also activates JNK/SAPK through a mechanism involving autocrine
stimulation of the purinoceptors (Hamada et al., submitted for publication). The activation of JNK/SAPK induced by either external stress signals or mitogen depletion was reported to lead to apoptosis [72, 73]. The direct phosphorylation of p53 by JNK/SAPK might be involved in this process [74]. However, several lines of evidence suggest that under certain conditions JNK/SAPK, together with ERKs, constitutes the mitogenic signalling pathway downstream of Ras (see below).

In addition to MAPK/ERKs and JNK/SAPKs, PI3K represents another important signalling molecule that is activated by Ras. PI3K is a heterodimeric complex composed of an 85 kDa regulatory subunit (p85) with SH2 regions and a 110 kDa catalytic subunit (p110) [75]. The specific binding of p85 SH2 regions to a consensus phosphotyrosine-containing sequence, either in the cytoplasmic domain of the growth factor receptors or in the receptor substrates [76], as well as the direct interaction between p110 and Ras [37], act in concert to recruit PI3K to the plasma membrane and to activate it to a maximal extent (Fig. 2). In addition, recent studies showed involvement of the src family of tyrosine kinases [77] and the rho family of small GTP-binding proteins [78, 79] in the activation of PI3K. A series of mutation analysis of PDGFβ receptor revealed that the critical importance of the PI3K-binding tyrosine residues (Y740 and Y751) in the cytoplasmic domain of the PDGFβ receptor for PDGF-induced mitogenesis [80, 81]. Indeed, studies adopting the microinjection of anti-PI3K antibodies and putative inhibitors for PI3K demonstrate that activation of PI3K is required for G1/S progression in response to growth factors [82–84] (Fig. 2). In addition, PI3K is implicated in other functions of the cell where protein sorting and membrane trafficking are involved. Candidate downstream effectors of PI3K include atypical PKC isoforms which do not bind either Ca²⁺, 1,2-diacylglycerol or phorbol esters. PKCζ has been reported to be activated by phosphatidylinositol-3,4,5-trisphosphate [85], a putative second messenger produced by PI3K. It has also been suggested that PI3K acts as an upstream regulator for several other protein kinases including p70S6K and PKB (c-Akt) [70, 83, 86–88] (Fig. 2), although there are some studies arguing against this view [69, 89]. The 70 kDa S6 kinase, which is a physiologically relevant kinase responsible for phosphorylation of the ribosomal 40S subunit S6 protein (Fig. 2), is critically involved in mitogenic signal transduction [67, 90]. Its upstream regulator, implicated as the target of the rapamycin/FKBP12 complex (Fig. 2), has been cloned (variously termed FRAP, RAFT or mTOR) and turned out to possess a strong sequence similarity to the lipid kinase domain of PI3K p110 [89].

**MPF, Cyclin and cdc2**

In 1971, Masui and Markert [91] first reported that the cytoplasm of maturing Xenopus oocytes contained an activity that could induce meiotic maturation when injected into immature oocytes. This activity, originally called the maturation promoting factor (MPF), was subsequently demonstrated in a wide range of eukaryotic cells including oocytes, zygotes and somatic cells when the cells were undergoing transition from the interphase to either the meiotic or mitotic metaphases (i.e., the G2 to M phase transition) [92–94]. Therefore, MPF also stands for M-phase promoting factor, in recognition of its wider role.) In addition, MPF was capable of inducing G2/M transition in a cell-free system, as evidenced by such characteristic features as nuclear envelope breakdown, chromosome condensation and spindle formation [95–97]. The potency of MPF, when assayed either by microinjection into immature oocytes or by the in vitro system, showed a periodic rise and fall during the course of early meiotic maturation, as well as that of early embryonic cell cycles, suggesting that cycling of the MPF activity is an integral part of the oscillator that controls the cell cycle [93, 95–101]. Lohka et al. [102] purified MPF to a homogeneity and found that it was composed of two components with molecular masses of 34 and 45 kDa.

Another series of investigations demonstrated that G2/M transition is associated with a burst of phosphorylation of nuclear proteins, including histone H1 [103], nucleaseplasm [104], nuclear lamins [96, 105] and many other unidentified proteins [106, 107]. In addition, a protein kinase activity, called “growth-associated” histone H1 kinase or “M phase–specific” histone H1 kinase, was universally demonstrated in dividing cells, including mitotic Physarum [103, 108], meiotic starfish [109], amphibian oocytes [110, 111] and mitotic sea urchin eggs [112], as well as mitotic mammalian somatic cells [113–115]. Importantly, the protein kinase activity fluctuated during meiotic maturation and early embryonic cell cycles, exactly in the same way as MPF [106–116].

The third line of investigation in marine eggs disclosed the existence of proteins whose abundance oscillated dramatically along with progression through the cell cycle [117]. The proteins, termed cyclins, were synthesized and accumulated continuously through the interphase, and were then abruptly destroyed by proteolysis during the metaphase-anaphase transition [117–122], coinciding with the timing of in-
activation of M phase–specific histone H1 kinase. A significant role for cyclins in the regulation of cell division was implicated by the observations that the microinjection of synthetic mRNA made from either a clam or a sea urchin cyclin cDNA could stimulate immature frog oocytes to enter the M phase [118, 122]. This effect of cyclin mRNA was also demonstrated for mitotic cycles in the cell-free system [121]. Therefore, cyclin synthesis appeared to be essential for entry into mitosis, while the destruction of cyclins during mitosis also appeared necessary to exit from the M phase.

In parallel with discoveries in the higher eukaryotes of MPF, M phase–specific histone H1 kinase and cyclins, yeast geneticists isolated a panel of temperature-sensitive cell division cycle (cdc) mutants in an attempt to identify crucial molecules involved in cell cycle control. cdc2− was one of such mutant of the fission yeast Schizosaccharomyces pombe, which was arrested at two major control points during the mitotic cell cycle at a restrictive (raised) temperature, one at “START,” which is the checkpoint in the G1 phase where the cell becomes committed to traverse across the G1/S border, and the other in the G2 phase before the G2/M transition [123, 124]. Nurse’s group [125] and the group led by Beach [126], who used to work for Nurse, almost coincidentally cloned the cDNA of a human homologue of cdc2 that could complement the defect in cdc2− mutant. The deduced amino-acid sequences, which showed a 63% identity between S. pombe and H. sapiens, predicted that cdc2 encodes an evolutionarily conserved protein kinase. Indeed, by using either anti-cdc2 protein antibodies or anti-peptide antibodies raised against the PSTAIRE sequence EGVPSTAIRELKE (an amino-acid sequence represented by the single letter code) that is perfectly conserved among S. pombe cdc2, budding yeast Saccharomyces cerevisiae CDC28 [127] and human CDC2, they identified a ubiquitously expressed 34 kDa protein with a protein kinase activity toward histone H1 [125, 126].

At this point (in 1988–1989), several laboratories independently achieved the same conclusions [128–132]. First, MPF is the same molecular entity as the M phase–specific histone H1 kinase. Second, CDC2 is identical to the 34 kDa catalytic subunit of the kinase MPF. Third, the other 45 kDa subunit of MPF corresponds to a cyclin, whose binding to the catalytic p34CDC subunit (at the PSTAIRE-containing motif) is an essential prerequisite for activation of the kinase.

**Cyclin-Dependent Kinases (CDKs)**

**Drive Cell Cycle Transitions**

It is now established that the cyclin B/CDC2 complex plays a central role in G2/M transition in all eukaryotes, including those in yeasts and humans [133–135]. Its substrates include histone H1, lamin B, nucleolin, Cdc25C, pRb, RNA polymerase II, vimentin, caldesmon, pp60^src^ and many others [136, 137]. In yeasts, a single kinase (cdc2 and the homologue CDC28 in fission and budding yeasts, respectively) drives both the G2/M transition as well as other cell cycle transitions by association with different cyclin molecules. In higher eukaryotes, by contrast, a number of CDC2-related, cyclin-dependent kinases (CDKs) have been identified [138–142]. Accumulating evidence indicates that the CDKs in complexes with different cyclins pursue specified roles at distinct phases of the cell cycle through phosphorylation of their cognate substrate proteins (Fig. 3).

One of the crucial substrates of G1 cyclin-dependent kinases, including CDK4 in the complex with D-type cyclins (cyclins D1, D2 and D3) [143] and cyclin E/CDK2 [144], is Rb protein (pRb), which is the product of the retinoblastoma susceptibility gene, and was the first tumor suppressor gene identified [145]. In familial retinoblastoma, young children develop bilateral, multifocal tumors of the retina as the result of inactivation of both alleles of the Rb gene on chromosome 13q14, one by germ line mutation and the other acquired after birth. Survivors of familial retinoblastoma have an increased risk of developing a second neoplasm, typically mesenchymal in origin. Loss-of-function mutations of the Rb gene are also observed among sporadic cancers and sarcomas. A recent investigation has disclosed that pRb plays a pivotal role in the regulation of the G1 to S phase progression in

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Fig. 3. A cyclin-dependent kinase complexed with a cyclin drives the progression of a specific phase of the cell cycle. The level of p27^Kip1^ CDK inhibitor (CKI) is a critical determinant for the timing of activation of G1 cyclin–dependent kinases, including CDK4 complexed with D-type cyclins and cyclin E/CDK2.
normal cells and that the function of pRb is regulated by phosphorylation [144, 146]. Thus, during the G0 and G1 phases, pRb is in an un- or underphosphorylated active state and binds to E2F family transcription factors [147]. The pRb/E2F complex negatively regulates the expression of a number of E2F consensus sites in their promoter regions [148]. Cyclin D1/CDK4 becomes activated around the mid G1 phase [143], followed by activation of cyclin E/CDK2 late in the G1 phase [149, 150] (Fig. 3), resulting in the accumulation of increasingly phosphorylated, inactive forms of pRb. This causes the release of E2F family transcription factors which are in complexes with DP-1 [151], which induces the expression of S-phase genes by positive regulation through E2F-binding sites (see Fig. 5). Overexpression of either cyclin D1 or cyclin E shortens the G1 phase, whereas either antisense inhibition of their expression or the microinjection of specific antibodies causes G1 arrest [152–157]. It is also known that abrogation of the functions of cyclin A prevents entry into the S phase [158, 159]. pRb remains in the hyperphosphorylated inactive state until the end of M phase after entry into the S phase [144, 146], through which period both cyclin A/CDK2 and cyclin A-B/CDK2 are thought to catalyze the phosphorylation reaction [160, 161]. Additional members of Rb family proteins or ‘pocket’ proteins, including p107 and p130, are also implicated in controlling G1/S transition.

The activity of each CDK is tightly controlled by a series of multiple regulatory mechanisms, which act in a temporally well orchestrated manner to ensure the timely activation of individual CDKs (Fig. 4). They include: (1) synthesis of the activator subunit cyclin and its association with the CDK catalytic subunit; (2) essential phosphorylation on a conserved residue in the CDK subunit (Thr161 in CDC2 (≡CDK1), Thr160 in CDK2 and Thr172 in CDK4), a reaction catalyzed exclusively by CDK-activating kinase (CAK), which itself turned out to be a cyclin-dependent kinase (i.e., cyclin H/CDK7 complex); (3) activation of CDK dephosphorylation by a dual specificity phosphatase (Cdc25) that removes phosphate moieties from Thr14 and Tyr15, which correspond to the ATP-binding region of CDK; and (4) degradation of cyclin by a ubiquitin-dependent process and inactivation of CDK. It is worth noting that there exists a positive feed forward mechanism for entry into the M phase, in which active cyclin B/CDK2 phosphatases and activates Cdc25C, an activator of cyclin B/CDK2 itself [162] (Fig. 4). A similar mechanism has also been suggested for G1/S progression, where cyclin E/CDK2 activates Cdc25A by phosphorylation [163, 164].

In addition to the mechanisms depicted in Fig. 4, several CDK inhibitor (CKI) proteins play crucial roles, particularly in the regulation of G1 cyclin-dependent kinases [165, 166] (Table 1). They are classified into two classes on the basis of both structural and functional characteristics. The CKIs of the first
Table 1. Two classes of CDK inhibitors (CKI).

<table>
<thead>
<tr>
<th>CKI</th>
<th>Target molecules</th>
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<tr>
<td>p21Waf1/Cip1/Sdi1</td>
<td>Various cyclins and CDKs (except for CAK), preferring cyclin/CDK complex</td>
</tr>
<tr>
<td>p27Kip1</td>
<td>CDK4 and CDK6 only</td>
</tr>
<tr>
<td>p57Kip2</td>
<td>CDK4 and CDK6 only</td>
</tr>
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group, including p21Waf1/Cip1/Sdi1, p27Kip1, and p57Kip2, bind to a variety of CDKs and cyclins (except for CAK), preferentially to cyclin/CDK complexes rather than monomeric forms, interfere physically with the CAK-mediated activating phosphorylation of CDKs, and also inhibit preformed active (i.e., phosphorylated by CAK) cyclin/CDK complexes [166]. Several laboratories cloned p21Waf1/Cip1/Sdi1 nearly simultaneously by employing different strategies, which included cDNA cloning in search of wild-type p53-activated fragment (Waf1), for CDK-interacting protein (Cip1) and senescent cell-derived inhibitor (Sdi1) [165]. As mentioned earlier, the tumor suppressor p53 directly induces the expression of p21 by binding to a p53-binding site located in the promoter region of the p21 gene [167]. Thus, the induction of p21 is likely a mechanism for p53-mediated G1 arrest and tumor suppression. In addition, p21 is induced by p53-independent mechanisms as well. For example, a skeletal muscle-specific transcriptional regulator (MyoD) induces p21 in a p53-independent manner, leading to p21-mediated inhibition of the G1 cyclin-dependent kinases that are responsible for the phosphorylation and inactivation of pRb [168]. pRb, in its active underphosphorylated state, is known to be required for G0 arrest and differentiation. In addition to its role as a CKI, p21 has been shown to block DNA replication by direct interaction with proliferating cell nuclear antigen (PCNA), an essential DNA replication protein [169]. However, p21 does not inhibit the PCNA-dependent nucleotide-excision repair of DNA [170]. Indeed, DNA damage, produced by either ultraviolet irradiation or other causes, leads to an increase in the level of p53, resulting in p21-mediated cell cycle arrest in the G1 phase, which persists until DNA repair is completed. It has been proposed that p21 plays an important role under such conditions as terminal differentiation [171] and cell senescence [172]. By contrast, another p21-related CKI, p27 kinase inhibitor protein 1 (p27Kip1) has been implicated as a critical negative determinant for G1/S transition (Fig. 3). The timing of activation of G1 cyclin-dependent kinases, including cyclin Ds/CDK4 and cyclin E/CDK2, depends on the levels of both the active cyclin/CDK complexes and p27Kip1 CKI. Growth stimulation of quiescent cells results in gradual increases in the level of G1 cyclins, which is followed by association with CDK subunits. The activity of CAK is relatively constant throughout the cell cycle [173]. However, p27Kip1 prevents activation of either of the G1 cyclin/CDK complexes until the level of p27Kip1 gradually declines to the threshold level through mechanisms involving both translational regulation [174] and ubiquitin-dependent degradation [175]. In this respect, it has been proposed that the cyclin Ds/CDK4 complex has a dual role: it stoichiometrically titrates p27Kip1 CKI to allow subsequent activation of cyclin E/CDK2, and phosphorylates and inactivates the crucial substrate pRb [166]. Recently, p27Kip1 has been shown to be required for restriction (R) point control [176]. The R point is a mammalian cell cycle checkpoint located late in the G1 phase and is analogous to the yeast "START" checkpoint. Beyond this point, cells become independent of external growth stimuli and proceed autonomically through the rest of the G1 phase and enter the S phase [177]. p27Kip1, together with p21Waf1/Cip1/Sdi1, is also implicated in anchorage (attachment)-dependent growth, a typical feature of normal cells (except for blood cells) but not transformed cells [178]. In addition, studies on knockout mice have provided compelling evidence for their cell growth regulatory roles in vivo.

The second group of CKIs consists of several inhibitor for CDK4 (INK4) proteins which selectively bind to either CDK4 or CDK6 and prevent their association with D-type cyclins [166]. The genes of p16INK4a and p15INK4b are tandemly linked within 30 kb on chromosome 9p21, which is a region of common cytogenetic abnormalities frequently found in transformed cells [179]. Therefore, they are also referred to as multiple tumor suppressor 1 (MTS1) and MTS2, respectively. The enforced expression of INK4 inhibitors causes G1 arrest only when pRb is intact, indicating that the phosphorylation of pRb on residues specific for CDK4 (and possibly CDK6) is critical for G1/S progression.

**The Ras Signalling System Activates G1 Cyclin–Dependent Kinases through Multiple Mechanisms**

The molecular mechanisms by which mitogenic stimuli induce the activation of G1 cyclin–dependent kinases are not well understood. We have studied the
roles of the Ras signalling system in the activation of cyclin D1/CDK4 using an inducible expression system for a dominant negative (DN-) Ras mutant, Ras(Asn17) (Fig. 5). As the result of Ser to Asn replacement at position 17, Ras(Asn17) shows a much reduced affinity for GTP and a comparable affinity for GDP as compared to wild-type Ras, leading to the sequestration of Ras-GEF and inhibition of the activation of endogenous Ras in a dominantly negative manner [180]. NIH3T3(M17) is a cell line in which Ras(Asn17) is induced to be expressed in response to glucocorticoid treatment [181]. It was previously reported that the addition of dexamethasone to quiescent cells 8 h before the addition of epidermal growth factor (EGF) completely eliminated the EGF-induced initiation of DNA synthesis in NIH3T3(M17) cells but not in parental NIH3T3 cells [181]. We found that cyclin D1 was the principal D-type cyclin in NIH3T3(M17) cells, and that EGF-induced increases in the mRNA and protein levels of cyclin D1 were strongly inhibited by the expression of DN-Ras. Under the same conditions, the activation of CDK4 as measured by immune complex in vitro kinase assay was completely eliminated. On the other hand, when a DN- form of either MEK1 or MAPK was introduced, it inhibited EGF-induced DNA synthesis and activation of the cyclin A promoter activity almost entirely, just like DN-Ras expression. Since the expression of DN-Ras inhibits the EGF-induced activation of both 44 kDa MAPK/ERK1 and 42 kDa MAPK/ERK2, it was concluded that the MAPK/ERK pathway conveys the necessary mitogenic signal required for G1/S progression. To further elucidate the molecular mechanism for EGF-induced expression of cyclin D1, we examined the effects of expression of the DN-Ras and DN- forms of MEK1 and MAPK on cyclin D1 promoter activity. The EGF-stimulated increase in cyclin D1 promoter activity was inhibited completely by the expression of either DN-Ras, DN-MEK1 or DN-MAPK. In contrast, the expression of a constitutively active form of MEK1 markedly stimulated the cyclin D1 promoter activity of quiescent cells, and also rescued the inhibition by DN-Ras expression. The EGF-stimulated increase in cyclin A promoter activity was inhibited almost entirely by the expression of DN-CDK4, indicating that the activation of CDK4 is a prerequisite for the induction of cyclin A, which is a hallmark of progression into the S phase. These composite results indicate that Ras-dependent activation of MAPK/ERKs early in the G1 phase leads to transcriptional upregulation of cyclin D1, resulting in CDK4 activation and G1/S progression (N. Takuwa et al., manuscript in preparation) (Fig. 5). In support of this view are the observations that transformation induced by activated Ras is associated with the increased expression of cyclin D1 and acceleration of G1 progression [182, 183]. In addition, it was reported recently that transforming Ras mutants (Ras(Val12) and Ras(Leu61)) and c-Ets-2 activate the cyclin D1 promoter [184].

We further tried to examine the role of Ras in cell cycle progression late in G1 phase since we obtained the following findings. When dexamethasone is added up to 3 h but not 14 h after the addition of EGF, it still strongly inhibits the initiation of DNA synthesis, which starts approximately 10 h after the addition of EGF. Under this condition, the activation of CDK4 as well as CDK2 is eliminated completely. However, differently from the case in which dexamethasone was introduced 8 h before the addition of EGF as described above, EGF-induced increases in the mRNA and protein levels of cyclin D1 are not inhibited. The levels of other D-type cyclins (cyclin D2 and cyclin D3) did not show a detectable change in response to EGF or DN-Ras expression. These findings strongly
suggest that the inhibition of cellular Ras activity exclusively late in the G1 phase inhibits the activation of CDK4 through a mechanism other than inhibiting the expression of cyclin Ds. Rather, we found that the expression of DN-Ras late in the G1 phase prevents downregulation of p27Kip1 inhibitor, the process which normally takes place during the late G1 phase and is required for passage through the late G1 checkpoint (i.e., R point) (see above). In contrast, the level of p21Waf1/Cip1 did not show any increase in response to DN-Ras expression. The addition of boiled cell extract obtained from arrested cells (in which DN-Ras is expressed late in the G1 phase) to the cell extract of growing cells and preculture at 37°C before immunoprecipitation and the kinase assay revealed that arrested cells contain a heat-stable inhibitor for CDKs. The immunodepletion of arrested cell extract with anti-p27Kip1 antibodies resulted in complete cessation of CDK inhibitor activity. These results provide compelling evidence that the cellular Ras activity late in the G1 phase is a determinant for the protein level of p27Kip1 CKI and thus for passage through the R point and progression into the S phase (N. Takuwa, manuscript in preparation) (Fig. 5). The involvement of Ras in progression through the late G1 phase into the S phase was also demonstrated by Stacey and colleagues [185]. They showed that the microinjection of a neutralizing anti-Ras antibody either before or several hours after the addition of serum similarly inhibited DNA synthesis. Since the EGF-induced activation of MAPK/ERKs is transient and returns to the basal unstimulated level within 3 h, it is strongly suggested that a Ras effector(s) other than the MAPK/ERK pathway mediates the effect of Ras late in the G1 phase. Further studies are required to elucidate the mechanism for Ras-dependent downregulation of p27Kip1.

It was recently demonstrated [186, 187] that enforced expression of the rho family of small-molecular-weight G proteins stimulated quiescent cells to enter the S phase. These molecules including Rho, Rac1, and Cdc42 have been implicated in growth factor- or oncogenic Ras-induced organization of the actin cytoskeleton, such as the assembly of actin stress fibers and formation of membrane ruffles and filopodia. It is worth noting that Rac1 and Cdc42 stimulated DNA synthesis and induced transformation without activation of the Raf-MEK-MAPK/ERK pathway [186]. Instead, they stimulated JNK/SAPK [186], raising the possibility that the JNK/SAPK pathway conveys mitogenic signals.

We have recently found that the activation of Gα12 induces G1/S transition through mechanisms involving activation of both the ERK- and JNK/SAPK-signalling pathways (Mitsui et al., submitted for publication). Gα12, in its wild-type form, was originally isolated as an oncogene by expression cloning from the human cDNA library of a primary sarcoma [188]. Analyses of cDNA and amino-acid sequences have revealed that Gα12 is distantly related to other known Gα subunits (i.e., Gαs, Gαq and Gα13) and thus constitutes, together with Gα13, the fourth class of Gα proteins. The biological activity of Gα12 has not been fully elucidated thus far. However, accumulating evidence suggests that Gα12 is involved in pertussis toxin-insensitive, GPCR agonist-induced cell growth and transformation. First, the overexpression of even wild-type Gα12 resulted in neoplastic transformation in the presence of serum growth factors [188]. Second, the S-phase progression of astrocytoma cells stimulated by thrombin or serum (the major mitogenic activity of which is contributed to LPA) but not basic fibroblast growth factor, was abrogated by the microinjection of anti-Gα12 antibody [189]. We evaluated the roles of MAPK/ERK and JNK/SAPK signalling cascades in Gα12-induced G1 to S-phase cell cycle progression in NIH3T3(M17) cells. The transient expression of a GTPase-deficient, constitutively active mutant of Gα12, Gα12(R203C), resulted in increases in the number of S-phase cells over the vector control under serum-deprived conditions. Consistent with the ability of Gα12(R203C) to induce G1/S transition, its expression led to increases in cyclin A promoter activity, as well as E2F-mediated transactivation as measured with luciferase reporter vectors. Wild-type Gα12 showed similar stimulatory effects on cyclin A promoter activity and E2F-mediated transactivation, although to a lesser magnitude. We observed a modest but constitutive activation of MAPK/ERKs in cells transfected with Gα12(R203C), which was eliminated by dominant negative (DN-) Ras expression. Gα12(R203C) also induced a 4-fold increase in JNK activity, which was eliminated by DN- forms of either Rac1 or Ras. The expression of DN- forms of either Ras, MAPK, Rac1 or JNK inhibited Gα12(R203C)-induced increases in S-phase cells. Also, the DN- forms of the Ras, MAPK and JNK strongly inhibited Gα12(R203C)-induced stimulation of cyclin A promoter activity. These results demonstrate that both the Ras-MAPK and the Ras-Rac1-JNK pathways convey necessary, if not sufficient, mitogenic signals induced by Gα12 activation.

**Involvement of the Ca2+ Messenger System in Cell-Cycle Regulation**

The Ca2+ messenger system plays pivotal roles in the regulation of a variety of physiological functions including endocrine/exocrine secretion, muscle contrac-
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tion and neurotransmission. The cell cycle transitions are also subjected to regulation by the Ca²⁺ messenger system [190, 191 and references therein].

It is well established that increases in the intracellular free Ca²⁺ concentration ([Ca²⁺]) play an essential role in fertilized eggs to exit from meiotic metaphase arrest and initiate early embryonic cell cycles. Thus, the fertilization of mature eggs in a variety of species, including the sea urchin, *Xenopus* as well as mammals, induces a transient Ca²⁺ mobilization or a Ca²⁺ wave from intracellular Ca²⁺ stores, which originates from the site of sperm attachment and traverses across the entire egg cytoplasm, reaching to the opposite side of the egg. In addition, in mammalian eggs, a Ca²⁺ wave is followed by repetitive increases in [Ca²⁺], or Ca²⁺ oscillations, which depends on Ca²⁺ influx from outside of the cells. Inositol-1,4,5-trisphosphate (IP₃) and cyclic ADP-ribose are both implicated in the Ca²⁺ release mechanisms [192]. Recently, a 33 kDa soluble sperm protein was identified as the Ca²⁺ oscillation inducer. The protein, termed oscillin, is specifically localized at the equatorial segment of the sperm head, and its nucleotide sequence is reported to show similarity with a prokaryotic hexose phosphate isomerase [193]. The mechanism by which a rise in [Ca²⁺], induced either by fertilization or by parthenogenic stimuli, causes a metaphase to anaphase transition in arrested eggs remained elusive until recently. By employing a constitutively active truncated form of calmodulin (CaM)-dependent protein kinase II (CaMKII (1–290)) and the autoinhibitory domain CaMKII (281–303) as molecular tools, Lorca *et al.* [194] demonstrated that CaMKII mediates Ca²⁺-induced cyclin degradation and thus inactivation of MPF/cdc2. It is possible that CaMKII is involved in metaphase to anaphase transitions in somatic cells as well, since a transient rise in [Ca²⁺], is reported to occur at the onset of anaphase in kidney epithelial cells. Prior to the report by Lorca *et al.*, Baitinger *et al.* [195] demonstrated the involvement of CaMKII in nuclear envelope breakdown at G2/M transition in early embryonic cells of fertilized sea urchin eggs, by employing a similar autoinhibitory domain peptide. In addition, studies with CaMK-specific inhibitors strongly suggest that the S/G2 transition represents an additional candidate target for CaMK-mediated regulation [196].

On the other hand, Ca²⁺/CaM-regulated protein phosphatase 2B or calcineurin (CN) plays an important role in the cell cycle of T lymphocytes during the G0/G1 transition. The engagement of antigen with the cell surface T-cell receptor (TCR) of quiescent T cells leads to the activation of *src* family non-receptor-type protein tyrosine kinases, which leads to Ca²⁺ mobilization by both PLCγ-mediated production of IP₃ and direct binding and phosphorylation of the IP₃ receptor/Ca²⁺ channel; the latter resulting in sensitization of the IP₃ receptor to IP₃ [197]. Ca²⁺ in a complex with CaM activates CN, which dephosphorylates and activates a component of NF-AT, a T-cell specific transcription factor that is involved in gene expression of a crucial T-cell growth factor, interleukin (IL)-2. Immunosuppressants FK506 and cyclosporin A bind to their cellular target proteins (immunophilins), FKBP (FK506 binding protein) and cyclophilin, respectively. (Rapamycin and FK506 share the same target, yet they exert different actions.) The complexes, FK506/FKBP and cyclosporin A/cyclophilin, both directly bind to and inhibit CN, eliminating the antigen-stimulated clonal expansion of T cells [198].

It is widely recognized that the stimulation of quiescent cells with a variety of mitogenic stimuli, including those acting through growth factor receptor protein tyrosine kinases and seven transmembraneGPCRs, induces IP₃-mediated, transient mobilization of Ca²⁺ from intracellular stores, by activating phospholipase Cγ (PLCγ) and PLCβ, respectively. The rise in [Ca²⁺], at the G0/G1 border may in part contribute to the induction of an immediate early gene c-fos through CaMK-mediated phosphorylation of a transcription factor Ca²⁺/cyclic AMP response element binding protein (CREB). However, in cell types other than lymphocytes, the role of the [Ca²⁺], transient at the G0/G1 border remains elusive in the context of G1/S transition. Indeed, a combination of A23187 and a phorbol ester mimics the antigen stimulation of T cells, whereas, in fibroblasts, Ca²⁺ ionophores do not show any mitogenic effect either alone or in combination with mitogens. However, we found, in fibroblasts, that thapsigargin, which is a specific inhibitor of the endoplasmic reticulum Ca²⁺ pump and depletes IP₃-sensitive Ca²⁺ stores, inhibits G1/S transition completely when introduced to cells either before the addition of mitogens or just before the G1/S boundary, by inhibiting the expression of cyclin A and the activation of CDK2 [199]. These results suggest that the Ca²⁺ filling state inside of IP₃-sensitive stores, which include (1) endoplasmic reticulum where proteins are synthesized and processed and (2) inside of the nuclear envelope of which the outer leaflet possesses an IP₃ receptor/Ca²⁺ release channel, is critical for G1/S transition.

In addition to Ca²⁺ filling of the IP₃-sensitive stores, Ca²⁺ influx across the plasma membrane is essential for G1/S progression [190, 200–202] (Fig. 6). When the extracellular calcium concentration
Fig. 6. Roles of the Ca\(^{2+}\) messenger system in the regulation of G1/S progression. Ca\(^{2+}\) and calmodulin (CaM) are critically important for progression through the late portion of the G1 phase in non-transformed cells. In addition, Ca\(^{2+}\) mobilization at the G0/G1 border contributes to gene expression, which is especially important in T-cell receptor-mediated induction of interleukin-2. Protein kinase C (PKC) performs dual cell-cycle regulatory roles, depending on both the stage within the cell-cycle and PKC isoforms. GFR/PTK, growth factor receptor/protein tyrosine kinase; GPCR, G-protein coupled receptor.

([Ca\(^{2+}\)]\(_0\)) is reduced, cells from a variety of tissues, with notable exceptions for parathyroid cells and keratinocytes, fail to initiate DNA synthesis in response to growth factors or GPCR agonists. In sharp contrast, cells transformed by various means proliferate in a manner independent of [Ca\(^{2+}\)]\(_0\), and there is an inverse correlation observed between the ability of transformed cells to form tumors in vivo and their calcium dependence in cultures [190]. We attempted to elucidate the site and the mechanism of action of Ca\(^{2+}\) influx in G1-to-S phase cell-cycle progression. In a simplified model system of Swiss mouse 3T3 fibroblasts, a GPCR agonist bombesin/gastrin releasing peptide (GRP) stimulated DNA synthesis in the absence of other mitogens in a manner absolutely dependent on both protein kinase C (PKC) and [Ca\(^{2+}\)]\(_0\) [200]. When the [Ca\(^{2+}\)]\(_0\) was reduced, bombesin-induced increases in Ca\(^{2+}\) influx via a voltage-independent Ca\(^{2+}\) channel [22] were decreased with strong inhibition of bombesin-induced DNA synthesis. The addition of non-selective Ca\(^{2+}\) channel blocker cobalt to extracellular media in the presence of a normal [Ca\(^{2+}\)]\(_0\) also dramatically inhibited bombesin-induced DNA synthesis. However, mitogenic signalings elicited by bombesin early in the G1 phase were not at all inhibited by either reducing the [Ca\(^{2+}\)]\(_0\) or adding cobalt. These include: (1) the production of P\(_2\), (2) the transient mobilization of Ca\(^{2+}\) from intracellular stores, (3) an increase in the absolute amount of 1,2-diacylglycerol, (4) the activation of PKC as measured in vivo by phosphorylation of the endogenous substrate MARCKS protein, (5) the activation of ERKs and S6 kinase, (6) the expression of immediately early gene c-fos, and (7) the stimulation of glucose transport. Therefore, it was suggested that the molecular target of Ca\(^{2+}\) influx is located relatively late in the G1 phase. Subsequent studies in human umbilical vein endothelial cells and human diploid fibroblasts provided further evidence for this notion [201, 202]. Indeed, a reduction in the [Ca\(^{2+}\)]\(_0\) only during the later half of the G1 phase was sufficient to inhibit the initiation of DNA synthesis. In contrast, a reduction in the [Ca\(^{2+}\)]\(_0\) after cells have entered the S phase had no inhibitory effect on the ongoing DNA synthesis. Restoration of the [Ca\(^{2+}\)]\(_0\) rescued arrested cells to initiate DNA synthesis after a certain lag period. Similarly, the addition of active CaM antagonists, but not inactive analogues, inhibited the initiation of DNA synthesis when introduced to cells during the late portion of the G1 phase, as well as when added earlier. These findings indicate that Ca\(^{2+}\) derived from outside of the cells in complexes with CaM is a crucial element in mitogenic signal transduction late in the G1 phase and/or at the G1/S boundary [201]. We further tried to address the underlying molecular mechanisms for the regulation of G1/S progression by Ca\(^{2+}\) and found that growth factor-induced pRB phosphorylation and CDK activation in normal cells, but not in their transformed counterparts, were sensitive to the reduction of [Ca\(^{2+}\)]\(_0\) and CaM antagonists [202]. In addition, we found that growth factor-induced increases in the mRNAs of cyclin E, cyclin A, CDC2, CDC2K and E2F1 were inhibited by the reduction of [Ca\(^{2+}\)]\(_0\) and CaM antagonists. In sharp contrast, increases in the mRNA levels of cyclin D1 and CDK4 were barely affected by the reduction of [Ca\(^{2+}\)]\(_0\) or CaM antagonists (N. Takuwa et al., unpublished observations). Therefore, it is likely that the activation process of G1 cyclin–dependent kinases at a step downstream of cyclin D1 expression is the prime target of Ca\(^{2+}\)/CaM action.

These composite results demonstrate that Ca\(^{2+}\) and CaM are critically required for progression of the cell cycle at multiple steps including G0/G1, G1/S, S/G2 and G2/M.

In addition to CaM, accumulated evidence indicates that members of the protein kinase C (PKC) family play key roles in the signal transduction of cell growth and proliferation, regulating cell-cycle transitions in both positive and negative directions (Fig. 6). The direction of PKC-mediated regulation appears to depend on the PKC isoform involved and the phase within the cell cycle [203, 204]. To describe details on this topic...
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is beyond the scope of this review and, therefore, is not dealt with in this article.

Summary and Perspectives

The proliferation of a cell is the result of multiple rounds of cell-cycle progression, which consists of an ordered sequence of transitions from one state to the next. A recent investigation revealed that an evolutionarily conserved family of protein kinases, termed cyclin-dependent kinases (CDKs), drives eukaryotic cell-cycle transitions. In yeasts, a single CDK (cdc2 in fission yeast and its homologue, CDC28, in budding yeast) conducts both G1/S and G2/M transitions. In higher eukaryotes including humans, in contrast, multiple CDKs play specialized roles in the cell cycle at distinct phases. Thus, CDK4 and CDK6 in complexes with D-type cyclins govern progression through the mid to late G1 phase, when a transient rise in the cyclin E/CDK2 activity just before the G1/S border triggers entry into the S phase, followed by a sustained increase in the cyclin A/CDK2 activity, which directs progression through the S phase. Cyclin B/CDK2 (=CDK1) plays a central role in the initiation of both the mitotic and meiotic M phases. The activity of CDKs is tightly regulated by three major mechanisms: the expression of activating subunits cyclins, the phosphorylation/dephosphorylation of CDKs and changes in the levels of CDK inhibitor proteins (CKIs). Mitogens and antiproliferative signals exert regulatory influences on all of these mechanisms.

When quiescent cells at the G0/G1 interface are activated by external mitogenic stimuli, on many occasions, these stimuli activate the small molecular-weight GTP binding protein Ras, through receptor protein tyrosine kinases, G protein-coupled receptors or cytokine receptors. Active Ras in the GTP-bound form in turn interacts with effector molecules, leading to the activation of several important downstream pathways including the MAPK (ERK and JNK) pathways and PI3K. We and others demonstrated that both the ERK and JNK pathways are involved in the regulation of cyclin expression. Very recently, we also found that Ras activity regulates the expression level of the CDK inhibitor p27kip1, although the downstream mechanism of Ras-dependent regulation has yet to be resolved. Many of the mitogens activate the Ca2+ messenger system, which is also involved in the regulation of CDK activity and cell-cycle progression. These observations indicate that different information flows triggered by mitogen-stimulated receptor activation converge upon the regulation of CDK activity via several distinct mechanisms. Further studies are definitely required to elucidate an entire understanding of the regulatory mechanism for cell-cycle progression.

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