A

B

Fig. 2. ATM/ATR and CHK1/CHK2 kinases mediate the signaling network of the DNA damage and DNA replication checkpoints. (**A**) Phosphorylation target proteins of ATM and ATR kinases. There are two parallel pathways that respond to DNA damaging stress in mammalian cells. The ATM pathway responds to the presence of DSBs acting at all phases of the cell cycle. The ATR pathway not only responds to DSBs but also to the agents that disturb the function of replication forks. Following their activation by DSBs or replication stress, ATM/ATR kinases phosphorylate unique (red and black, respectively) or overlapping (green) target proteins at specific serine (S) or threonine (T) residues of indicated (if known) numbers. (**B**) Phosphorylation target

CACGTG (E-box) when dimerized with Max, another bHLH-ZIP. A head-to-tail pair of Myc-Max dimers form a heterotetramer that is capable of bridging distant E-boxes. Mitogen exposure promptly induces the expression of c-*myc*. Ectopic expression of c-*myc* also encourages quiescent cells to enter into S phase *(102)*. Myc not only targets genes that encode cyclins D2, D1 and E, and Cdc25A as a transcription factor, but also sequesters p27^{KIP1} into CDK4(6)/cyclin D complexes away CDK2/cyclin E to cause phosphorylation and subsequent ubiquitination and proteasome-mediated degradation of the p27^{KIP1}, thereby realizing at least three distinct regulatory functions of CDK2/Cyclin E activity, E2F-dependent transcription, and cell growth *(103)*.

In association with Max, Myc binds to the E-boxes in a variety of gene promoters and thus orchestrates the transcriptional activation of a diverse set of genes. However, Myc on its own inhibits the transcription of other genes, including *p21WAF1 (104)* and another cyclin-dependent kinase inhibitor, *p15INK4b (105,106)*. The DNA-binding protein Miz-1 directly recruits Myc to the *p21WAF1* promoter, where Myc selectively inhibits bound p53 from activating *p21WAF1* transcription and favors the initiation of apoptosis *(107)*. Thus, Myc can influence the outcome of a p53 response in favor of cell death.

2.2. The p53-pRB Pathway Controls the G1/S Transition

The *p53* tumor suppressor gene (TP53) is the most frequently mutated gene (about 50%) in human tumors, and encodes a 53 kDa transcription factor (p53) that directly induces the expression of a substantial number of genes that are important for cell cycle regulation, DNA damage repair, and apoptosis *(108,109)*. Of the genes that are induced by p53, p21^{WAF1} plays a pivotal role in G1 arrest by inhibiting CDK4(6)/cyclin D1 activity, thereby reducing the phosphorylation of pRB and promoting G1 arrest of the cell cycle. This interconnecting signaling pathway involving p53, pRB, and E2F plays an essential role in G1/S transition of the cell cycle. p21^{WAF1} is also known to inhibit S phase progression (G1 arrest) by binding to PCNA, a ring protein that promotes DNA replication *(63)*. The expression level of p53 is low in the absence

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Fig. 2. *(continued)* proteins of CHK1 and CHK2 kinases. CHK2 is primarily phosphorylated by ATM (and partially by ATR), whereas CHK1 is phosphorylated by ATR. Then, CHK1/CHK2 kinases transmit the checkpoint signals by phosphorylating unique (red and black, respectively) or overlapping (green) target proteins at specific serine (S) or threonine (T) residues of indicated numbers. CHK2, phosphorylated on Thr-68 by ATM, is activated to autophosphorylate on Thr-383 and Thr-387 (blue arrows), further enhancing its kinase activity. Ser-46 of p53 is presumed to be phosphorylated by putative p53 S46 kinase. These phosphorylated proteins further propagate the signal to the downstream targets, thereby regulating various cellular events.

of cellular stress. However, various types of stress, including DNA damage, induce p53 expression and cause G1 arrest. In cases where the DNA damage is too severe to be repaired, p53 induces apoptosis as a desperate attempt to protect the organism *(19,110, 111)*. This essential role of p53 as a critical brake on tumor development explains why it is so frequently found in cancer cells *(112,113)*.

Other genes that are upregulated by p53 *(112)* include *cyclin G1 (27)*, *MDM2* (murine double murine 2), *BAX* (bcl2-associated X protein), *GADD45 (114)*, *14-3-3σ (115)*, *CDK4 (116)*, *p53R2 (117,118)*, *p53AIP1 (119)*, *p53DINP1 (120)*, and *p53RDL1 (121)*. Cyclin G1 and MDM2 regulate the stability of the p53 protein (*see* **Subheading 2.3.**). Bax forms a homodimer or heterodimer with Bcl2, and increasing amounts of the Bax homodimer trigger cytochrome-c release from mitochondria, thus promoting apoptosis *(122)*. Gadd45 (induced after growth arrest and DNA damage) is involved in regulating nucleotide excision repair of UV-damage together with p53 and another p53-downstream gene, *p48XPE (123)*. The 14-3-3σ protein associates with and recruits Cdc25C from the nucleus to inhibit the activation of CDK1/cyclin B, thus causing G2 arrest. p53R2 is a homolog of ribonucleotide reductase small subunit (R2). Expression of p53R2, but not that of R2, is induced by DNA damage and serves to supply the cell with the deoxyribonucleotides needed for DNA repair. p53RDL1 (p53-regulated receptor for death and life) interacts with its ligand Netrin-1 and promotes the survival of damaged cells against apoptosis.

At least some of the eleven phosphorylation sites identified on p53 seem to play pivotal roles in its regulation. Three functionally important domains have been identified in the p53 molecule, and phosphorylation at these sites is considered to influence the structural changes of these domains. The middle domain constitutes the core domain that associates with the specific nucletotide sequences at the promoter regions of its target genes. This domain harbors the vast majority of the p53 "hot spot" mutations found in human cancers. In cells with damaged DNA, Ser-15 of p53 is phosphorylated by ATM (ataxia telangiectasia mutated) or ATR (ATM-Rad3-related) *(124–126)*. The ATM gene was first isolated from patients with the autosomal recessive disorder ataxia telangiectasia (A-T). These patients exhibit cerebellar degeneration, immunodeficiency, radiation sensitivity, and predisposition to cancer *(127)*. Phosphorylation of p53 at Ser-20 by CHK1 or CHK2 may also be important for regulating the interaction between p53 and MDM2 *(128,129)*. Upon severe DNA damage, Ser-46 on p53 is phosphorylated and apoptosis is induced. As p53AIP1 (p53-regulated apoptosis-inducing protein 1) is selectively induced by p53 molecules that have been phosphorylated at Ser-46, it may be that p53AIP1 mediates this p53-dependent apoptosis by inducing the release of cytochrome-c from mitochondria *(130)*. p53DINP1 (p53-dependent damage-

nducible nuclear protein 1) functions as a cofactor of the putative p53-Ser46 kinase that promotes phosphorylation of p53 at Ser-46 *(120)*.

2.3. Regulation of p53 Stability by ARF and MDM2

The *INK4a* locus that generates p16^{INK4a} also encodes a degenerated gene product called ARF (after alternative reading frame) *(131)*. Thus, the locus encodes two tumor suppressor proteins, p16^{INK4a} and p19^{ARF} (p14^{ARF} in humans), which activate the growth suppressive functions of pRB and p53, respectively *(67)*. ARF is a highly basic (pI > 12), arginine-rich nucleolar protein *(132)*. Deletion of the *ARF* gene can inactivate p53 function in tumors where p53 itself remains intact. Transcription of the *ARF* gene is regulated by E2F, and thus the *INK4a/ARF* locus influences both the pRB-E2F and p53 pathways.

Overexpression in the same tumor lines of MDM2 (murine double murine 2; Hdm2 in humans), a protein whose expression is upregulated by p53 (*see* **Subheading 2.2.**), has the same effect. This is because ARF binds to MDM2 and abrogates its p53-inhibitory activity. MDM2 destabilizes p53 by catalyzing its ubiquitination by acting as an E3 ubiquitin ligase. This promotes the nuclear export of p53, thereby allowing it to be targeted for proteasomal degradation *(133,134)*. Actually, MDM2 is frequently overexpressed in human tumors, and this leads to the rapid degradation of p53 *(135)*. Since MDM2 directly binds to the N-terminus of p53, phosphorylations of p53 at Ser-15, Ser-20, and Thr-18 are important for the dissociation of MDM2 from p53 *(128,129)*. MDM2 is itself transcriptionally activated by p53, which thus creates a negative feedback loop. Consequently, inhibiting the interaction between p53 and MDM2 by the application of synthetic molecules may serve as an effective cancer treatment because it may lead to cell cycle arrest or apoptosis in p53-positive tumor cells *(109)*.

Other proteins also modulate MDM2 activity. Mitogen-induced activation of phosphatidylinositol 3-kinase (PI3-kinase) and its downstream target, the AKT/PKB serine-threonine kinase, results in the phosphorylation of MDM2 on Ser-166 and Ser-186. CDK2/cyclin A also phosphorylates MDM2 on Thr-216 *(136)*. These phosphorylation events are necessary for the translocation of MDM2 from the cytoplasm into the nucleus and thus serve to promote the p53-inhibitory activity of MDM2 as a ubiquitin ligase *(134)*. Cyclin G1 directly binds to MDM2 *(137)*, recruits PP2A (protein phosphatase 2A) to dephosphorylate MDM2 at Thr-216, and releases MDM2 from p53, thereby cooperating with ARF to restrict the ability of MDM2 to negatively regulate p53 *(138)* (**Fig. 1**). Indeed, cyclin G1$^{-/-}$ mouse embryo fibroblasts show enhanced accumulation of p53 and are partially deficient in an irradiation-induced G2/M-phase checkpoint *(139)*. Cyclin G2 may also have redundant or compensatory

functions, because it associates with many of the same proteins to which cyclin G1 binds, including p53, PP2A, MDM2, and ARF *(137)*. This p53-stabilizing effect of PP2A/cyclin G complexes may also influence the malignancy of cancer cells, considering that enhanced expression of a truncated form of PP2A was observed in highly metastatic melanoma cells *(140)*. Cells overexpressed with this truncated form of PP2A show irradiation-induced checkpoint defects and appear to elevate genetic instability, which may promote tumor progression *(141)*. These data suggest that cyclin G1 is a positive feedback regulator of p53, since it downregulates the activity of MDM2, which would otherwise restrain the accumulation of p53 *(142)*.

3. DNA Damage Checkpoints

DNA damage caused by IR, chemical reagents, or similar environmental insults induces cell cycle arrest at G1, S, or G2, thereby preventing the replication of damaged DNA or aberrant mitosis until the damage is properly repaired. The molecular mechanism in mammalian cells that detects the presence of double-strand breaks (DSBs) is not well understood. Research in the budding yeast *Saccharomyces cerevisiae,* however, tells us that a quintet complex composed of RAD24, RFC2, RFC3, RFC4, and RFC5 acts in this organism as a sensor of DSBs *(143)*. Disruption of these components causes defects in the damage checkpoint machinery of *S. cerevisiae (144)*. The same DSB-sensing mechanism is also used in another useful yeast strain, *Schizosaccharomyces pombe (145,146)*. In *S. cerevisiae,* the signal of the DSB abnormality is transmitted to the ring-shaped hetero-trimer that is composed of Ddc1/Rad17/Mec3 (Rad9/Rad1/Hus1 in fission yeast and mammals). This hetero-trimer resembles the replication factor PCNA *(147)*. In fission yeast, this complex activates Rad3 kinase, which then phosphorylates CHK1 *(148)*. The activated CHK1 then targets Cdc25C for phosphorylation. Cdc25C is subsequently recognized by Rad24, a 14-3-3 protein (*see* **Subheading 2.2.**), which recruits it out from the nucleus into the cytoplasm, where it inactivates the CDK1/cyclin B complex (Cdc2/Cdc13 in *S. pombe*), which results in G2/M arrest *(149)*. The 14-3-3 proteins bind to serine/threonine-phosphorylated residues in a specific manner and regulate key proteins involved in various physiological processes such as the cell cycle, intracellular signaling, apoptosis, and transcription regulation *(150)*. Similar checkpoint regulatory mechanisms involving 14-3-3 proteins are also employed in vertebrate cells *(2,86,151)*. For example, human CHK1 is activated by phosphorylation and thereby phosphorylates Cdc25C on Ser-216, which is recognized by the 14-3-3σ protein. The14-3-3σ protein then removes Cdc25C from the nucleus to the cytoplasm, thereby preventing the activation of the CDK1/cyclin B complex and entry into mitosis *(3,152–154)*.

In mammalian cells, there are two parallel pathways that respond to DNA-damaging stresses (**Fig. 2**). The first pathway is the ATM pathway, which responds to the presence of DSBs at all phases of the cell cycle. The second pathway is the ATR (ATM-Rad3-related) pathway, which responds not only to DSBs but also to the agents that interfere with the function of replication forks *(126,4,127)*. A third pathway may involve the newly identified ATX (ATM-related X protein), which phosphorylates and activates CHK1 and/or CHK2 *(126,127,155)*. As shown in **Fig. 2**, ATM phosphorylates many target proteins at their specific serine or threonine residues and activates their functions. In response to IR, for example, ATM phosphorylates RAD9 on Ser-272 *(156)*; PLK3, which further phosphorylates CHK2, contributing to its full activation *(157)*; SMC1 (the cohesin protein) on Ser-957 and Ser-966 *(158,159)*; H2AX on Ser-140, which is required for 53BP1 accumulation at DNA break areas *(160)*; 53BP1 on Ser-6, Ser-25/Ser-29, and Ser-784 *(161)*; and MDC1 *(162)*.

The human ATR protein complexes stably with a protein called ATRIP (ATR-interacting protein). These complexes localize in nuclear foci after damage and thus appear to be recruited to the sites of DNA damage *(163)*. The ATR homologs in fission yeast (Rad3) and budding yeast (Mec1) also form similar complexes with the ATRIP-related factors Rad26 and Ddc2/Lcd2/Pie1, respectively, which are also recruited to the sites of DNA damage *(164–167)*. ATR phosphorylates H2AX on Ser-139 *(168)*, whereas ATM/ATR phosphorylate E2F on Ser-31 *(169)*. The checkpoint functions of ATM in response to IR are primarily mediated by the effector kinase CHK2, whereas those of ATR in response to replication inhibition and UV-induced damage are mediated by CHK1. Thus, the structurally unrelated CHK2 and CHK1 proteins channel the DNA damage signals from ATM and ATR, respectively *(21,170)*. However, recent observations suggest the existence of various "crosstalks" among these kinases *(100,171)*, and the presence of a novel checkpoint cascade signaling by way of ATM-CHK1 to Tousled-like kinases (TLKs) that causes chromatin remodeling in response to various stresses *(172)*.

The expression of the labile CHK1 protein is restricted to the S and G2 phases *(173)*. Although it is active even in unperturbed cell cycles, it is further activated in response to DNA damage or stalled replication *(100,174)*. Following a checkpoint signal, CHK1 is phosphorylated on Ser-317 and Ser-345 by ATR in cooperation with the sensor complexes, which include the mammalian homologs of Rad17 and Hus1. The phosphorylation at the Ser-345 site is required for nuclear retention of CHK1 following an HU-induced checkpoint signal *(175–177)*. CHK1 not only stimulates the kinase activity of DNA-dependent protein kinase (DNA-PK) complexes, which leads to increased phosphorylation of p53 on Ser-15 and Ser-37; it also elevates the DNA-PK-

dependent end-joining reactions, thereby promoting the repair of DSBs *(178)*. *CHK1⁻/⁻* mice show a severe proliferation defect and death in embryonic stem (ES) cells and peri-implantation embryonic lethality. The ES cells lacking CHK1 have also been shown to have a defective G2/M DNA damage checkpoint in response to IR *(179,180)*. In contrast, CHK1-deficient cells called DT40 are viable, but they fail to arrest at G2/M in response to IR and fail to maintain viable replication forks when DNA polymerase is inhibited *(181)*.

In contrast, the other Ser/Thr protein CHK2 kinase (also known as hCds1) must be phosphorylated at Thr-68 by ATM to activate it in response to IR-induced DNA damage (this is not the case for damage owing to UV or HU) *(170,182)*. Unlike CHK1, CHK2 is a stable protein that is expressed throughout the cell cycle and that seems to be inactive in the absence of DNA damage *(173)*. Its activation involves its dimerization and autophosphorylation. Unlike the catalytically inactive form of CHK2, wild-type CHK2 leads to G1 arrest after DNA damage by phosphorylating p53 on Ser-20, which causes the preformed p53/MDM2 complexes to dissociate and increases the stability of p53 *(128)*. Unlike *ATM⁻/⁻* and *p53⁻/⁻* mice, *CHK2⁻/⁻* mice do not spontaneously develop tumors, although the IR-induced G1/S cell cycle checkpoint—but not the G2/M or S phase checkpoints—was impaired in primary mouse embryonic fibroblasts (MEFs) derived from *CHK2⁻/⁻* mice *(183,184)*.

That the fission yeast homolog of CHK2, Cds1, may participate in repair is suggested by the finding that it interacts with the Mus81–Eme1 endonuclease complex, which can resolve the Holliday junction *(185,186)*. The human Mus81–Eme1 complex also has a similar function as a flap/fork endonuclease that is likely to play a role in the processing of stalled replication fork intermediates *(187)*.

CHK1 and CHK2 share partly redundant roles in that they target common downstream effector proteins such as the Polo-like kinase 3 (PLK3) *(188)*, the promyelocytic leukemia (PML) protein *(189)*, the E2F1 transcription factor *(190)*, or the TLKs *(172)*. PLK3 binds to and phosphorylates p53 on Ser-20. Through this direct regulation of p53 activity, PLK3 is at least partly involved in regulating the DNA damage checkpoint as well as M-phase function. The *PML* gene is translocated in most acute promyelocytic leukemias and encodes a tumor suppressor protein that plays a pivotal role in gamma irradiation–induced apoptosis. It is proposed that CHK2 mediates gamma irradiation–induced apoptosis in a p53-independent manner through an ATM-CHK2-PML pathway *(189)*. PML also recruits CHK2 and p53 into the PML-nuclear bodies, and enhances the p53/CHK2 interaction to protect p53 from MDM2-mediated ubiquitination and degradation *(191)*. Mutations in the prototypic member of the Tousled (Tsl) kinase from the plant *Arabidopsis thaliana* lead to a pleiotropic phenotype *(192)*. In mammals, however, the TLKs are regulated in a cell

cycle-dependent manner that peaks at S phase and are involved in chromatin assembly by phosphorylating the chromatin assembly factors Asf1a and Asf1b *(193)*. CHK1 phosphorylates TLK1 on Ser-695 in vitro, and substitution of Ser-695 with alanine impairs the efficient downregulation of TLK1 after DNA damage *(172)*.

4. G1 Checkpoint Response

In mid-to-late G1, and if the cellular environment is favorable for proliferation, a binary decision—whether to commit to the mitotic cell cycle and enter S-phase, or whether to not commit to the cell cycle and remain in a quiescent, non-proliferative state—is made at the "restriction point" *(194,195)*. As described above, many proteins are involved in making this critical decision and in ensuring proper progression of the G1/S transition. Although cyclin D meets the criteria of the critical restriction point factor, the system seems to be far more complex than just relying on a single factor. Moreover, the relationship between the restriction point and DNA damage checkpoints remains elusive *(196)*. The cell cycle checkpoints that monitor the proper G1/S transition and S phase progression during potentially hazardous genotoxic stress *(103, 197)* will be discussed in the following sections.

4.1. The ATM(ATR)/p53-Mediated Pathway

The ATM(ATR)/p53 pathway plays a pivotal role in one of the checkpoint mechanisms that arrest the cell cycle at G1 phase following DNA damage (G1 checkpoint) **(Fig. 3)**. As described in **Subheading 3**, ATM is activated in response to IR, whereas ATR is activated in response to replication inhibition or UV-induced damage. The activated ATM or ATR then phosphorylates p53 (on Ser-15), and this phosphorylation causes MDM2 to dissociate from p53, which stabilizes p53 and leads to its accumulation *(128,129)*. Increased expression of ARF owing to E2F1 stabilization in response to DNA damage also blocks the inhibitory function of MDM2, thereby increasing the nuclear amount of p53.

The principal kinases relaying ATM(ATR)-initiated checkpoint signaling are preferentially CHK2 for ATM and CHK1 for ATR. In response to IR or DNA replication stress, ATR phosphorylates CHK1 at Ser-317 and Ser-345 *(175,177,198)*, which moderately increases its kinase activity and allows it to propagate the signal to downstream effectors, including p53, which CHK1 phosphorylates on Ser-20 *(129)*. In response to IR, ATM phosphorylates CHK2 at Thr-68 *(199)*, followed by CHK2 autophosphorylation on Thr-383 and Thr-387 and the activation of several target proteins, including p53, which CHK2 also phosphorylates on Ser-20 *(128,129)*. These Ser-20 phosphorylation events both induce MDM2 to dissociate from p53.
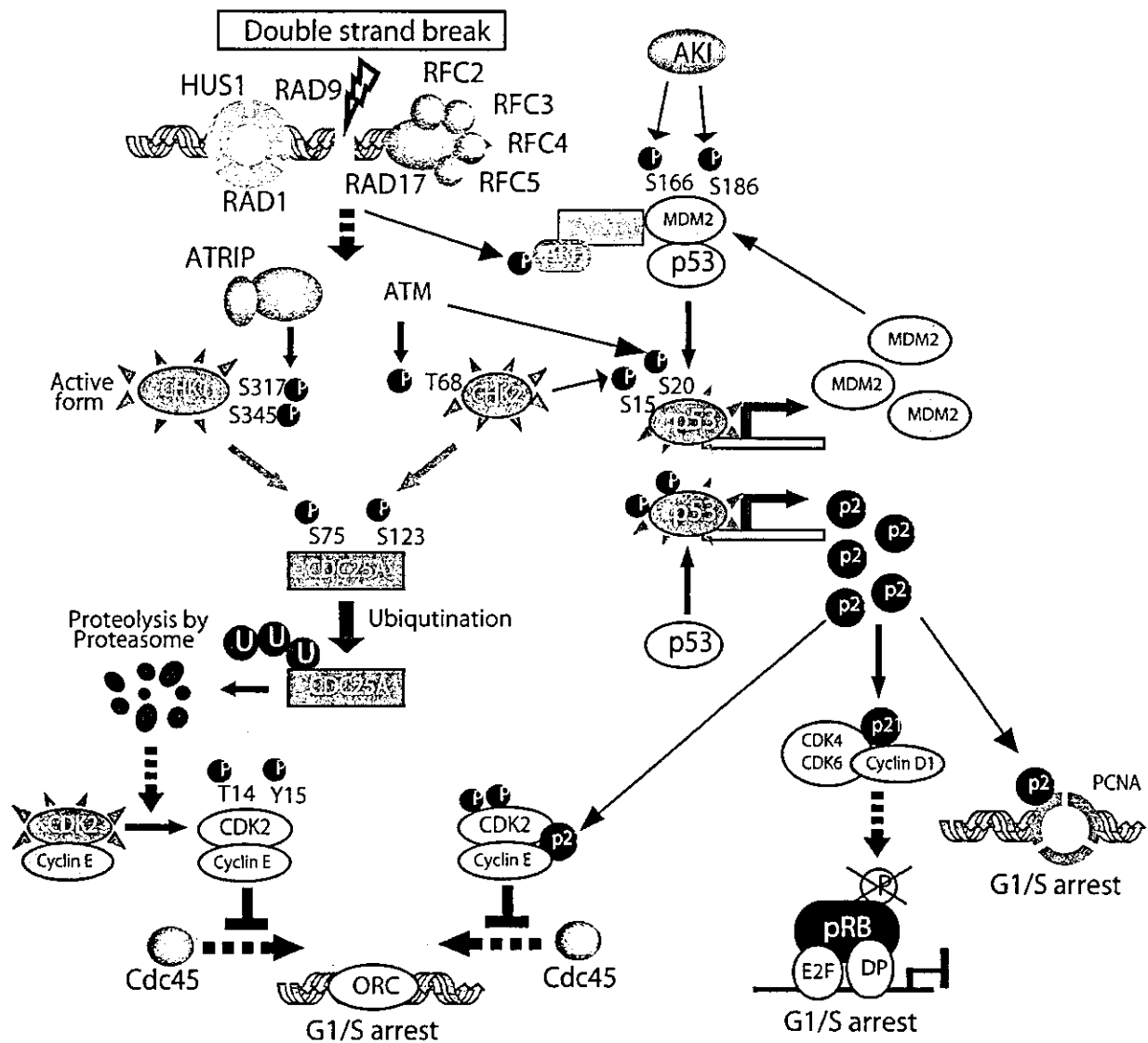
Fig. 3. ATM(ATR)-mediated G1/S checkpoint pathways. DNA damage triggers a rapid cascade of phosphorylation events involving either the ATM and CHK2 (upon IR) or the ATR and CHK1 (upon UV light) kinases. These phosphorylation events activate the target protein kinases to trap and phosphorylate the next target proteins, thereby transmitting the DNA damage signals. It has been determined that in response to IR, ATM phosphorylates CHK2 at Thr-68, whereas ATR (or ATM) phosphorylates CHK1 at Ser-317 and Ser-345. In one pathway (left), the CHK2 or CHK1 kinase phosphorylates Cdc25A phosphatase at serines 75, 123, 178, 278, and 292 (100). Of these, the Ser-123 residue that is targeted by CHK2 and the Ser-75 residue that is phosphorylated by CHK1 seem to be particularly critical residues of Cdc25A (202, 99). The phosphorylated Ser-123 or Ser-75 residue is recognized by the ubiquitination (Ub) enzyme, and this promotes the rapid degradation of Cdc25A by the proteasome. Due to the disappearance of Cdc25A phosphatase activity that this degradation causes, the CDK2/cyclin E complex is locked in its inactive form because of the presence of the inhibitory phosphorylation on the Thr-14 and Tyr-15 residues of CDK2. Thus, the CDK2/cyclin E complex fails to load Cdc45 onto chromatin and the blockade of the initiation of the DNA replication origins is maintained.

The stabilized and activated p53 protein that results from CHK1/CHK2-mediated phosphorylation induces the transcription of a large number of genes, including $p21^{WAF1}$, which silences the kinase activities of the CDK2/cyclin E, CDK2/cyclin A, or CDK4(6)/cyclin D complexes. This prevents the complexes from loading the Cdc45 origin binding factor onto chromatin, which precludes the recruitment of DNA polymerases, thereby blocking initiation of DNA replication from the unfired origins *(200,201)*. Another important consequence of inhibiting both the CDK2 and CDK4(6) kinase complexes is that these complexes cannot then phosphorylate pRB, which allows pRB to maintain its inhibition of the E2F-dependent transcription of S-phase genes that are essential for S-phase entry as described in **Subheading 2.1.4.** These effects all result in G1 arrest. Maintenance of the G1/S arrest by way of this pathway after DNA damage is a delayed response that requires the transcription, translation, and/or protein stabilization of key checkpoint transducers. However, once initiated, this pathway provides a long-lasting G1 arrest, and the entry into S phase is prevented as long as a single unrepaired DNA lesion is detected by the checkpoint machinery.

4.2. The ATR(ATM)/Cdc25A-Mediated Pathway

The human Cdc25A phosphatase plays a pivotal role at the G1/S transition because it enhances the kinase activities of the CDK2/cyclin E and CDK2/cyclin A complexes by dephosphorylating the inhibitory phosphorylated Thr-14 and Tyr-15 residues of CDK2 *(102197)*. After UV and IR exposure, Cdc25A is ubiquinated because it is phosphorylated by CHK1 (in the case of UV)

Fig. 3. *(continued)* In the other pathway (right), Ser-15 of p53 is directly phosphorylated by ATM or ATR in cells with damaged DNA. The phosphorylation of p53 at Ser-20 by CHK1 or CHK2 induces the dissociation of the p53/MDM2 complex, which increases the stability of p53 because MDM2 primes p53 for ubiquitination and proteasomal degradation (*see* **Fig. 1**). CHK1 also stimulates the kinase activity of DNA-PK complexes, which increases the phosphorylation of p53 on Ser-15 and Ser-37. Furthermore, DNA damage can also upregulate ARF, which specifically inhibits MDM2, putatively in collaboration with the cyclin G proteins. The collective result is that stable and transcriptionally active p53 transcription factor accumulates in the cell nucleus and induces the expression of a large number of target genes, including the p21 CDK inhibitor. The increased p21 levels inhibit the CDK2/cyclin E complex or the CDK4(6)/cyclin D complexes, thus arresting the cell cycle at G1/S phase. Upon severe DNA damage, Ser-46 on p53 is phosphorylated by the putative p53-Ser46 kinase with the aid of p53DINP1, which selectively induces the expression of p53AIP1, which is a mediator of apoptosis because it induces the release of cytochrome-*c* from mitochondria.

(87,96,) and CHK2 (in the case of IR) *(202)*. The critical residue of Cdc25A that is targeted by CHK2 is Ser-123 *(202)*. Cdc25A is also phosphorylated on Ser-75 by CHK1 *(109)*. The phosphorylated Ser-123 residue (and possibly also the phosphorylated Ser-75 residue) is recognized by the ubiquitination (Ub) enzyme, which promotes the rapid degradation of Cdc25A by the proteasome system. Removal of Cdc25A in turn keeps the CDK2-associated kinase complexes in their inactive form due to the persisting inhibitory phosphorylation of their Thr-14 and Tyr-15 residues. This results in G1 arrest. The important target of this cascade is the inhibition of CDK2-dependent loading of Cdc45 onto DNA pre-replication complexes. Thus, the ATM(ATR)–CHK2(CHK1)–Cdc25A–CDK2 pathway accounts for the rapid and p53-independent initiation of the G1 checkpoint, where the abundance and activity of Cdc25A decreases without delay in response to IR- or UV-mediated DNA damage **(Fig. 3)** . It is likely that this regulatory mechanism is conserved among vertebrates and operates in every cell type.

During interphase, CDK2 appears to phosphorylate Cdc25A, which constitutes a Cdc25A-CDK2 autoamplification feedback loop *(203)*. Cdc25A also seems to be involved in the G2/M transition, besides its commonly accepted effect on G1/S progression *(87)*. Proteolysis of Cdc25A is also linked with the intra-S-phase checkpoint, which guards against premature entry into mitosis in the presence of stalled replication forks.

4.3. Other Potential G1 Checkpoint Pathways

It has been reported that there is another G1 checkpoint induced by IR exposure, which is characterized by enhanced protein degradation *(204)*. In this checkpoint, DNA damage unmasks a cryptic "destruction box" (RxxL) within the cyclin D1 amino-terminus that is then recognized by the anaphase-promoting complex (APC) ubiquitin ligase, which primes cyclin D1 for rapid proteasomal destruction *(197)*. This causes the p21^{WAF1} protein, which served as an assembly factor of the CDK4(6)/cyclin D1 complexes, to be released. p21^{WAF1} is then free to bind to another of its targets, the CDK2/cyclin E complex. This binding inactivates the kinase activity of the complex **(Fig. 3)** . Since the proliferation of many mammalian somatic cells depends on the presence of abundant CDK4(6)/cyclin D1 complexes, the destruction of cyclin D1 together with the inactivation of the S-phase-promoting CDK2/cyclin E strongly induces G1 arrest.

Exposure of epithelial cells to UV light can also lead to yet another G1 checkpoint mechanism. This mechanism involves the gradual accumulation of p16^{INK4a}, which selectively disrupts the CDK4(6)/cyclin D1 complexes. This again causes the release of p21^{WAF1}, which can then bind to and inhibit CDK2/cyclin E, thereby resulting in G1 arrest. If these mechanisms are confirmed as

cell cycle checkpoints, they would each serve as examples of an ATM-independent, cell-type-restricted response. Note that because cyclins D2 and D3 are not degraded upon DNA damage, these pathways would have little effect in cell types that express several D-type cyclins or lack cyclin D1.

To ensure the exact duplication of the genome during every cell division, which is a basic requirement of every proliferating cell, eukaryotes adopt a strategy that temporally separates the assembly of the pre-replication complex (pre-RC) from the initiation of DNA synthesis **(Fig. 4)** *(201,205)*. A key component of the pre-RC is the hexameric minichromosome maintenance (MCM) protein complex, which consists of the six Mcm2–Mcm7 proteins *(206)*. The MCM complex is presumed to be a helicase functioning in the growing forks, and like other helicase proteins *(207)*, it actually adopts a toroidal structure when observed under a microscope *(208)*. The MCM complex is recruited to the replication origins, where the two protein kinase complexes Cdc7–Dbf4 (in budding yeast) and CDK2/cyclin E trigger a chain reaction that results in the phosphorylation and activation of the MCM complex and finally in the initiation of DNA synthesis *(201,209)*. At the onset of S phase, S-phase kinases promote the association of Cdc45 with MCM at the origins. Upon the formation of the MCM–Cdc45 complex at the origins, the duplex DNA is unwound and various replication proteins, including DNA polymerases, are recruited onto the unwound DNA *(200)*. A "licensing checkpoint" that prevents passage into S phase in the absence of sufficient origin licensing may also exist in mammalian cells *(210)*.

5. The S-Phase Checkpoint

Proliferating cells are always exposed to life-threatening insults that disturb the proper replication and segregation of their genomes into daughter cells. In response to these genotoxic insults, eukaryotic cells have evolved checkpoint mechanisms that monitor the progression of DNA replication at S phase and halt replication if an abnormality is observed **(Fig. 4)** . At least two distinct S-phase checkpoints seem to exist. One of these occurs in response to DNA-replication stress that interferes with the proper progression of the replication forks. The other is an intra-S-phase checkpoint that functions in response to DSBs *(2,4)*. The S-phase checkpoint may also have a function during an unperturbed S phase, because even in the absence of exogenous agents, mutants of the many genes that are involved in this checkpoint show aberrant checkpoint signaling, and some mutants also cause checkpoint induction *(2)*.

5.1. S-Phase Checkpoint in Response to DNA Replication Stress

Several types of agents are known to interfere with the function of replication forks and to elicit the S-phase checkpoint. These include agents that
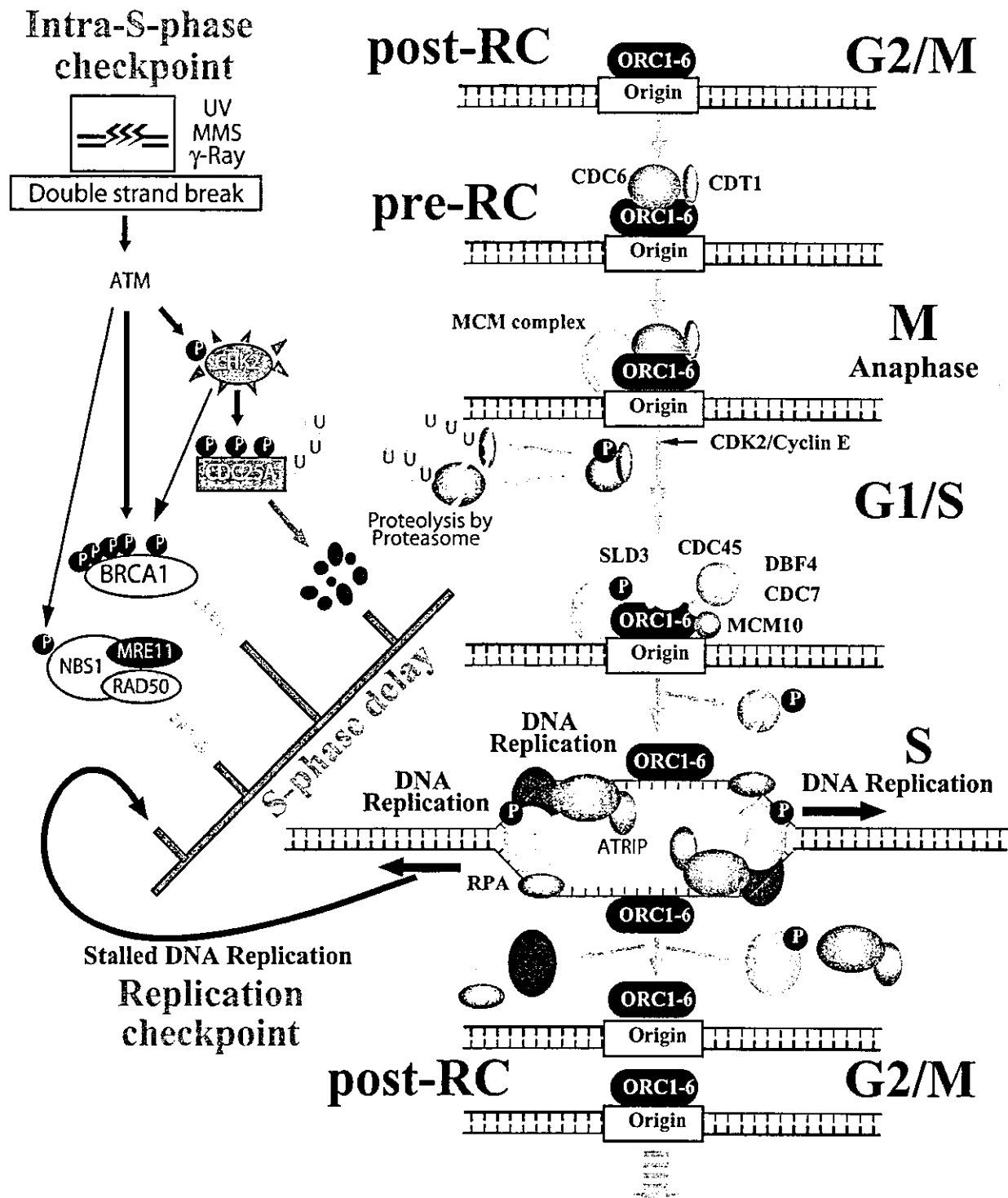
Fig. 4. Molecular mechanism of S-phase progression and the S-phase checkpoints. Upon initiation of DNA replication, DNA replication initiation factors such as MCM10, CDC45–Sld3 (budding yeast) and RPA and checkpoint complexes bind to the pre-replicative complex (pre-RC) on chromatin and trigger the unwinding of DNA. The hexameric MCM2-7 complex is recruited to the replication origins by a number of proteins, including MCM10, RPA, and CDC45–Sld3. The MCM complex is a putative helicase of the growing forks. Two protein kinase complexes, Cdc7/Dbf4 (budding yeast) and CDK2/cyclin E, trigger a chain reaction that results in the phosphorylation of the Mcm complex and finally the initiation of DNA synthesis. The

irectly inhibit DNA synthesis. For example, HU stalls replication forks by
epleting the deoxynucleotide triphosphate (dNTP) pool, while aphidicolin ac-
vates the checkpoint by inhibiting DNA synthesis by blocking the activities
f polymerases (**Fig. 4**) . In addition, DNA-modifying agents that block repli-
ation can elicit the S-phase checkpoint. These agents include methyl
ıethanesulfonate (MMS) and UV-induced DNA lesions, which slow down
ıe rate of DNA-replication-fork progression in budding yeast *(211)*.

The study of the DNA-replication checkpoint is most advanced in yeasts.
ıowever, the checkpoint mechanisms that were unveiled in yeasts also seem
) be conserved in mammalian cells *(2,149)*. The central checkpoint kinases
Iec1 (ATR in humans) and Rad53 (CHK2 in humans) play an essential role in
ıaintaining DNA replication fork stability in response to DNA damage and
ıplication fork blockage, and they inhibit the activation of late-firing replica-
on origins after HU and MMS exposure *(4)*. The DNA replication forks ap-
ɛar to function both as the activator and as the primary effector of the S-phase
ıeckpoint pathway, since the recruitment of Ddc2 (ATRIP in humans) to
uclear foci and the subsequent activation of the Rad53 kinase occurs only
uring S phase and requires the assembly of the replication forks *(212)*.

In budding yeast, proteins that are essential for DNA replication, such as
ıNA polymerase ε and its interacting partners Dpb11 and Drc1/Sld2, are also
ɛquired for efficient checkpoint activation *(213)*. Dpb11 and its human ho-
ıolog TopBP1 associate with the PCNA-like protein Ddc1 and human Rad9,

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ig. 4. *(continued)* ATR/ATRIP (Rad3/Rad26) complex and the Polα–primase com-
ex and several other replication proteins are also recruited to the unwound DNA.
fter this, the RAD1/RAD9/HUS1 complex binds to chromatin, an event that requires
e RAD17/RFC2-5 complex.

Two kinds of S-phase checkpoint mechanisms are known. One monitors the stalled
ɛplication forks (DNA-replication checkpoint) while the other monitors the replica-
ɔn block induced by DSBs during S phase (intra-S-phase checkpoint). In contrast to
e checkpoints at the G1/S and G2/M transitions that arrest the cell cycle, these S
ıase checkpoints can only delay the progression of S phase. Proteins involved in the
gulation of DNA replication such as DNA polymerase ε, Dpb11 (TopBP1 in hu-
ans), Drc1/Sld2 (budding yeast), Ddc1 (budding yeast), and RPA are also required
ır the S-phase checkpoint in response to replication blockage. Claspin (Mrc1 in yeast)
at is phosphorylated at Ser-864 and Ser-895 by CHK1 also regulates the S-phase
ıeckpoint. ATM is the master transducer of the S-phase checkpoint and phosphory-
tes BRCA1 and BRCA2 as well as NBS1 (at Ser-343), which is a component of the
BS1/MRE11/RAD50 complex. CHK1 also phosphorylates TLK (at Ser-695), a pro-
in kinase that is potentially involved in regulation of chromatin assembly. Acetyla-
ɔn of nucleosomal histone H3 or H4, which regulates the chromatin structure, and
ɛne expression also play a role in the S-phase checkpoint.

respectively, and seem to collaborate in monitoring the progression of replication forks *(214,215)*. The Polα–primase complex and RPA (replication protein A) are also required for the S-phase checkpoint in response to replication blocks *(216)*.

Claspin, a CHK1-interacting protein, is required for the ATR-dependent activation of CHK1 in *Xenopus* egg extracts that contain incompletely replicated DNA *(217)*. Claspin, ATR, and Rad17 bind to chromatin independently and appear to collaborate in checkpoint regulation by detecting different aspects of the DNA replication fork *(218)*. *Xenopus* Claspin may be phosphorylated at Ser-864 and Ser-895 by CHK1 *(219)*. Human Claspin is a cell cycle-regulated nuclear protein whose levels peak at S/G2 phase and that is phosphorylated in response to replication stress or other types of DNA damage. It appears to work as an adaptor molecule that brings the ATR/CHK1 and RAD9/RAD1/HUS1 complexes together to regulate the S-phase checkpoint *(220)*. These observations suggest that the activation of CHK1 by ATR may be regulated by Claspin in a similar way in budding yeast: Rad9 is phosphorylated by Mec1 in response to DNA damage and subsequently serves as a scaffold protein for Rad53, thus allowing Rad53 to autophosphorylate and self-activate *(221)*. Mrc1, a yeast homolog of Claspin, is also important for the activation of Rad53 and Cds1 in response to HU, and thus may mediate the checkpoint response to replication blockage in a similar manner to Claspin *(222,223)*.

In budding yeast, the S-phase checkpoint activates the ATM-like Mec1 and the CHK2-related Rad53 kinases in response to stalled replication forks that arise owing to replication stress or DNA damage in S phase. These kinases in turn inhibit spindle elongation and late origin firing, which stabilize the DNA polymerases at the arrested forks *(4)*. Orc 2 (origin recognition complex 2) plays a pivotal role in maintaining the number of functional replication forks, and the amount of DNA damage required for Rad53 activation is higher in S phase than in G2 *(224)*. For the S-phase checkpoint, acetylation of the nucleosomal histone H3 or H4 that regulates chromatin structure and gene expression also appears to be important *(225)*. Studies in fission yeast suggest that the signal activating the S-phase checkpoint is generated only when replication forks encounter DNA damage *(226)*.

5.2. S-Phase Checkpoint in Response to DSBs

After DNA damage, proliferating cells actively slow down their DNA replication by activating a checkpoint. This gives the cell time to repair the damage. This checkpoint is often called the intra-S-phase checkpoint (**Fig. 4**) *(4,21)*. The intra-S-phase checkpoint consists of regulatory networks that sense DNA damage and coordinate DNA replication, cell cycle arrest, and DNA repair.

The above-mentioned Cdc25A degradation pathway also appears to induce the transient intra-S-phase response. Here, IR-induced formation of DSBs triggers degradation of Cdc25A, which in turn inhibits the S-phase promoting activity of CDK2/cyclin E and induces the transient blockade of DNA replication, which delays S-phase progression for several hours *(227)*. As described above, Cdc25A destruction involves the phosphorylation of Cdc25A on Ser-123 by both CHK1 and CHK2 in response to IR, and on Ser-75 by CHK1 in response to UV irradiation *(99)*. Supporting the involvement in the S-phase checkpoint of ATM, its phosphorylation targets including CHK2, and the CHK2-regulated Cdc25A-CDK2 cascade, is the fact that mutants of ATM, CDK2, or the other proteins in the CHK2-regulated Cdc25A-CDK2 cascade fail to inhibit S-phase progression when they are irradiated. Consequently, these cells undergo radio-resistant DNA synthesis (RDS), which is a phenomenon of persistent DNA synthesis after irradiation *(127,199)*.

Another phosphorylation target of ATM, the master transducer of the S-phase checkpoint, plays a key role in the intra-S-phase checkpoint, namely, BRCA1 (breast cancer susceptibility gene 1). BRCA2 may also be an important target of ATM *(228,229)*. Mutations in the *BRCA1* and *BRCA2* tumor suppressor genes are responsible for the great majority of familial breast and ovarian cancers. These proteins form nuclear foci with Rad51 during S phase and after DNA damage *(230)*. *BRCA1-* and *BRCA2*-mutant cells exhibit defects in the homologous repair of chromosomal DSBs. *BRCA1* or *BRCA2* deficiency in mice results in early embryonic lethality, but conditional deletions reveal that mice with *BRCA1* or *BRCA2* mutations suffer a wide range of carcinomas *(231)*. Moreover, a mammary epithelium whose *BRCA1* or *BRCA2* gene has been deleted is highly susceptible to mammary tumorigenesis *(232)*. *BRCA1* is omnipresent and plays broad roles in transcriptional regulation that include both p53-dependent and -independent responses. It also has ubiquitin ligase activity when dimerized to Bard1, and undergoes damage-associated phosphorylation by multiple kinases that precedes repair-complex formation *(230)*. In contrast, *BRCA2* has a more straightforward function{\}it is central to homology-directed repair (HDR) because of its interaction with Rad51 and its direct binding to single-stranded DNA *(233)*.

Another important phosphorylation target of ATM that plays a role in the intra-S-phase checkpoint is NBS1 (Nijmegen breakage syndrome gene 1) *(234–36)*. NBS 1 (Xrs2 in yeast) forms a multimeric complex with the MRE11/RAD50 nuclease, MDC1 (mediator of DNA damage checkpoint protein 1), and other unidentified proteins, and recruits them to the vicinity of DNA damage sites by direct binding to the phosphorylated histone H2AX *(237)*. ATM phosphorylates NBS1 at Ser-343 in response to IR *(238)*. Cells harboring a point mutation of NBS1 at this phosphorylation site failed to engage in the S-

phase checkpoint induced by IR (239). Moreover, in collaboration with the BRCA1 C-terminus domain, the highly conserved NBS1 forkhead-associated domain plays a crucial role in the recognition of damaged sites (240). After recognizing the DNA damage, the NBS1 complex proceeds to rejoin the DSBs predominantly by homologous recombination repair in vertebrates. This process collaborates with the cell cycle checkpoints at S and G2 phase to facilitate DNA repair.

Mutations in the MRE11-complex genes result in sensitivity to DNA damage, genomic instability, telomere shortening, aberrant meiosis, and abnormal checkpoint signaling in S phase. Blockade of NBS1-MRE11 function and the CHK2-Cdc25A-CDK2 pathway entirely abolishes the inhibition of DNA synthesis that is normally induced by IR. This results in the complete RDS that is also seen when cells harbor a defective ATM gene (227). However, the phosphorylation of NBS1 and CHK2 by ATM seems to trigger two distinct branches of the intra-S-phase checkpoint because CDK2-dependent loading of Cdc45 onto replication origins, a prerequisite for the recruitment of DNA polymerase, is prevented in normal or NBS1/MRE11-defective cells when they are irradiated but not in irradiated cells that harbor a defective ATM protein (227). 53BP1, which plays a partially redundant role in the phosphorylation of the downstream checkpoint effector proteins BRCA1 and CHK2, is also a key transducer of the intra-S-phase and G2-M checkpoint arrests that occur in response to IR (241).

CHK1 may also be necessary for the intra-S-phase checkpoint when DNA synthesis is inhibited by DNA damage (242). Supporting this is that chemical or genetic ablation of human CHK1 triggers the accumulation of Cdc25A, prevents the IR-induced degradation of Cdc25A, and causes RDS (87). Moreover, the basal turnover of Cdc25A operating in unperturbed S phase requires CHK1-dependent phosphorylation of its Ser-123, Ser-178, Ser-278, and Ser-292 residues (100). The ATR-CHK1 pathway may also play an important role in the intra-S-phase checkpoint that is induced by replication-associated DSBs caused by application of the topoisomerase I inhibitor topotecan (TPT) (243), although it has no relationship with DNA-PK activity (244). However, in budding yeast, the intra-S-phase checkpoint control is not activated by another topoisomerase I inhibitor, camptothecin (CPT), and the CPT-hypersensitive mutant strain that fails histone 2A (H2A) Ser-129 phosphorylation is an essential component for the efficient repair of DSBs that do not induce the intra-S-phase checkpoint (245). In Xenopus egg extracts, DNA lesions generated by exonuclease or etoposide, a DNA topoisomerase II inhibitor, activate a DNA damage checkpoint that blocks the initiation of DNA replication (246). TLK, a protein kinase that is potentially involved in regulating chromatin assembly and that is phos-

phorylated by CHK1 on its Ser-695 residue, also appears to be involved in the ATM/CHK1-dependent intra-S-phase checkpoint *(172)*.

Besides its function with H2AX (a histone H2A variant), Mdc1 (mediator of DNA damage checkpoint protein 1) controls damage-induced checkpoints by promoting the recruitment of repair proteins to the sites of DNA breaks *(247)*. Cells that lack the *MDC1* gene are sensitive to IR because they fail to activate the intra-S-phase and G2/M checkpoints properly, probably due to an inability to regulate CHK1 properly. Thus, MDC1 facilitates the establishment of the intra-S-phase cell cycle checkpoint *(248)*. Notably, MDC1 is hyperphosphorylated in an ATM-dependent manner, and rapidly relocalizes to nuclear foci at sites of DNA damage, which appears to be crucial for the efficient activation of the intra-S-phase checkpoint *(249)*.

The ATR/ATRIP complex requires the RFC (replication factor C) and PCNA-like proteins to fully activate the replication-stress response because RFC recognizes the primer-template junction and recruits PCNA onto DNA to function as a sliding clamp that tethers DNA polymerases *(4,250)*. In fission yeast and humans, the PCNA-like complex (Rad1/Rad9/Hus1 or RAD1/RAD9/HUS1) is recruited in a RAD17-dependent manner onto the chromatin after damage *(149,251)*. In budding yeast, the homologous PCNA-like complex Rad17/Mec3/Ddc1) is recruited to DSBs and the sites of DNA damage in a Rad24-dependent manner *(252,253)*. Thus, it is possible that the Rad17 complex recognizes DNA damage and loads the PCNA-like complex onto DNA, thereby responding to DNA damage independently of ATR/ATRIP *(254)*.

As with fission yeast, RAD17 and HUS1 are required for the phosphorylation of CHK1 by ATR in mammals *(254,255)*. ATR also phosphorylates Rad17 at its Ser-635 and Ser-645 residues *(256)*. This phosphorylation is significantly stimulated by the increased amounts of PCNA-like complexes that were recruited onto the chromatin after damage. Unlike the *hus1*-null fission yeast cells, which are defective for the G2/M DNA-damage checkpoint, mouse cells that lack the mouse homolog of the fission yeast protein Hus1 enter mitosis normally after DNA damage but display an S-phase checkpoint defect *(257)*. The mouse Hus1 protein also seems to play a role in the NBS1-independent checkpoint-mediated inhibition of DNA synthesis that is generated by the genotoxin benzo(a)pyrene dihydrodiol epoxide (BPDE), which causes bulky DNA adducts. However, the *hus1*-null mouse cells displayed intact S-phase checkpoint responses in response to IR-induced DSBs *(257)*.

5. Defects in G1/S Checkpoint and Cancer

Defects in the genome maintenance mechanisms, including DNA repair and cell cycle checkpoint pathways, are believed to enhance genetic instability and

cause the accumulation of mutations and chromosomal aberrations that is a hallmark of cancer cells *(155)*. Most of the G1/S checkpoint transducers and effectors are classified as either tumor suppressors or proto-oncogenes, and their loss-of-function mutations or overexpression appear to play pivotal roles in many types of human tumors. Mouse models that mimic the defects of these genes display similar phenotypes to human patients, which suggests that these checkpoint regulators are important in the surveillance of genomic destabilization and the prevention of tumor development.

Mutations in the p53 gene are responsible for the large majority of sporadic human cancers, and thus p53 is a key target for cancer therapy *(67,108,110,135)*. p53 gene mutations can also be inherited in a subset of families with the Li-Fraumeni syndrome (LFS), which is characterized by a predisposition to sarcomas, brain and breast tumors, and childhood adrenocortical carcinoma *(258)*. The inactivation of the *INK4a/ARF* (or *CDKN2a*) locus, which engages the pRB and p53 tumor suppressor pathways through its capacity to encode the two distinct gene products p16^{INK4a} and p14^{ARF}, is also a common genetic event in the development of human melanoma *(259)*. Human cells harboring pRB and p53 mutations also cause telomere dysfunction that results in the chromosomal end-end joining and fusion-bridge-breakage cycles that trigger the aneuploidy observed in most cancer cells *(67)*. Both p53- and ARF-deficient mice spontaneously develop tumors and die of cancers early in life, and the primary MEFs cultured from p53- and ARF-deficient mice do not senesce in culture but instead yield immortal cell lines *(67)*. Moreover, many Burkitt lymphomas (BL) carry point mutations in the p53 tumor suppressor gene, bear other defects in the p14^{ARF}-MDM2-p53 pathway, or the p16^{INK4a} gene is inactivated by promoter methylation or homozygous deletion *(260)*. Thus, disruption of both the pRB and p53 pathways is also critical for BL development. Overexpression of cyclin E, which deregulates the G1/S checkpoint and contributes to genomic instability, is also observed in several types of human tumors, including carcinomas of the lung, breast, and head and neck *(21)*. Furthermore, overexpression of Cdc25A in a subset of breast cancers is associated with poor patient survival, which suggests that both Cdc25A and its downstream target CDK2 might represent suitable therapeutic targets in early-stage breast cancer *(261)*.

ATM is the gene responsible for the rare disorder A-T, which is a genomic instability syndrome that causes cancer predisposition, radiation sensitivity, neurodegeneration, and immunodeficiency. The cells of A-T patients show markedly abnormal cell cycle checkpoint responses at G1, S, and G2 *(127,199,262)*. Moreover, while LFS, the highly penetrant familial cancer phenotype, is usually associated with inherited mutations in the p53 gene, some LFS families that do not have germline mutations of p53 have instead het-

rozygous germline mutations in CHK2 *(258,263)*. This suggests that human CHK2 is a tumor suppressor gene whose mutation confers a predisposition to arcomas, breast cancers, and brain tumors. Supporting this is the fact that ocasional sporadic cancer-associated mutations have been detected in both the CHK1 and CHK2 genes *(263)*. In certain patients with an A-T-like disorder A-TLD), mutations in MRE11, but not in ATM, are found, and the clinical resentations of these patients mutated in *hMRE11* genes are virtually idential to those seen in A-T patients *(237,264)*.

Fanconi's anemia (FA) is an autosomal recessive disease that is characterzed by bone marrow failure, developmental anomalies, a high incidence of nyelodysplasia and acute nonlymphocytic leukemia, cellular hypersensitivity o crosslinking agents, and a high risk of developing acute myeloid leukemia nd certain solid tumors *(265,266)*. The six known FA gene products (FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG proteins) interact in a ommon pathway, in which the mono-ubiquitination and nuclear foci formaion of FANCD2 are essential. Mono-ubiquitinated FANCD2 colocalizes with BRCA1 and hRad51 in S-phase-specific nuclear foci *(265,267)*. ATM phoshorylates FANCD2 on its Ser-222 residue in response to IR, and this is required for the activation of an S-phase checkpoint. Thus, FANCD2 links the FA and ATM damage-response pathways *(268)*. Consequently, the FA proeins are involved in the cell cycle checkpoint and DNA-repair pathways, and lisruption of the FA genes results in chromosome instability, a common feaure of many human cancers *(232)*.

The *BRCA1* gene was cloned by positional cloning as one of the genes that onfers genetic predisposition to early-onset breast and ovarian cancer *(230)*. The *BRCA2* tumor-suppressor gene was also identified by a similar approach 230). Inherited mutations in *BRCA1* or *BRCA2* predispose people to develop reast, ovarian, and other cancers *(269)*. BRCA2 has been identified as being he seventh FA gene, and mutated BRCA2 protein fails to bind to Rad51 in esponse to genotoxic stress, which prevents Rad51 from localizing to nuclear lamage foci *(231,270)*. It has been suggested that thet FA proteins FANCA, BRCA2, and FANCD2 act indirectly with the cellular defense machinery gainst oxidative stress by linking it with the defense machinery against DNA lamage *(271)*.

Nijmegen breakage syndrome (NBS) is a recessive genetic disorder that is haracterized by elevated sensitivity to IR that induces DSBs and a high frequency of malignancies *(240)*. Cells derived from NBS patients show chromoome fragility, IR sensitivity, and RDS (failure to suppress S-phase progression n the presence of IR-induced DSBs) *(239)*. These phenotypic features are remiiscent of those in the cells established from A-T patients, although the clinical resentation of NBS differs considerably from that of A-T. RDS was first re-