Activation of ATR in response to replication stress

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20/12/2022

https://www.reactome.org
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 83

This document contains 1 pathway and 9 reactions (see Table of Contents)

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Genotoxic stress caused by DNA damage or stalled replication forks can lead to genomic instability. To guard against such instability, genotoxically-stressed cells activate checkpoint factors that halt or slow cell cycle progression. Among the pathways affected are DNA replication by reduction of replication origin firing, and mitosis by inhibiting activation of cyclin-dependent kinases (Cdks). A key factor involved in the response to stalled replication forks is the ATM- and rad3-related (ATR) kinase, a member of the phosphoinositide-3-kinase-related kinase (PIKK) family. Rather than responding to particular lesions in DNA, ATR and its binding partner ATRIP (ATR-interacting protein) sense replication fork stalling indirectly by associating with persistent ssDNA bound by RPA. These structures would be formed, for example, by dissociation of the replicative helicase from the leading or lagging strand DNA polymerase when the polymerase encounters a DNA lesion that blocks DNA synthesis. Along with phosphorylating the downstream transducer kinase Chk1 and the tumor suppressor p53, activated ATR modifies numerous factors that regulate cell cycle progression or the repair of DNA damage. The persistent ssDNA also stimulates recruitment of the RFC-like Rad17-Rfc2-5 alternative clamp-loading complex, which subsequently loads the Rad9-Hus1-Rad1 complex onto the DNA. The latter '9-1-1' complex serves to facilitate Chk1 binding to the stalled replication fork, where Chk1 is phosphorylated by ATR and thereby activated. Upon activation, Chk1 can phosphorylate additional substrates including the Cdc25 family of phosphatases (Cdc25A, Cdc25B, and Cdc25C). These enzymes catalyze the removal of inhibitory phosphate residues from cyclin-dependent kinases (Cdks), allowing their activation. In particular, Cdc25A primarily functions at the G1/S transition to dephosphorylate Cdk2 at Thr 14 and Tyr 15, thus positively regulating the Cdk2-cyclin E complex for S-phase entry. Cdc25A also has mitotic functions. Phosphorylation of Cdc25A at Ser125 by Chk1 leads to Cdc25A ubiquitination and degradation, thus inhibiting DNA replication origin firing. In contrast, Cdc25B and Cdc25C regulate the onset of mitosis through dephosphorylation and activation of Cdk1-cyclin B complexes. In response to replication stress, Chk1 phosphorylates Cdc25B and Cdc25C leading to Cdc25B/C complex formation with 14-3-3 proteins. As these complexes are sequestered in the cytoplasm, they are unable to activate the nuclear Cdk1-cyclin B complex for mitotic entry.

These events are outlined in the figure. Persistent single-stranded DNA associated with RPA binds
claspin (A) and ATR:ATRIP (B), leading to claspin phosphorylation (C). In parallel, the same single-stranded DNA:RPA complex binds RAD17:RFC (D), enabling the loading of RAD9:HUS1:RAD1 (9-1-1) complex onto the DNA (E). The resulting complex of proteins can then repeatedly bind (F) and phosphorylate (G) CHK1, activating multiple copies of CHK1.

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Stalling of DNA replication fork and RPA binding

Location: Activation of ATR in response to replication stress

Stable identifier: R-HSA-176175

Type: binding

Compartments: nucleoplasm

When a DNA replication fork encounters DNA lesions (e.g., cyclobutane pyrimidine dimers or alkylated bases) stalling of the replicative DNA polymerase may occur. This can lead to dissociation or 'uncoupling' of the DNA polymerase from the DNA helicase and generation of long regions of persistent ssDNA. Uncoupling can also occur in response to other genotoxic stresses such as reduced dNTP pools caused by hydroxyurea treatment which inhibits cellular ribonucleotide diphosphate reductase. The exposed ssDNA is bound by the single-stranded DNA binding protein RPA. The persistent nature of this RPA-ssDNA complex (as opposed to a more-transient complex found at an active replication fork) allows it to serve as a signal for replication stress that can be recognized by the ATR-ATRIP and Rad17-Rfc2-5 complexes.

RPA associates with ssDNA in distinct complexes that can be distinguished by the length of ssDNA occluded by each RPA molecule. These complexes reflect the progressive association of distinct DNA-binding domains present in the RPA heterotrimeric structure. Binding is coupled to significant conformational changes within RPA that are observable at the microscopic level. Presumably, the different conformations of free and ssDNA-bound RPA allow the protein to selectively interact with factors such as ATR-ATRIP when bound to DNA.

Followed by: Recruitment of Rad17-RFC complex to DNA, Binding of ATR-ATRIP to the RPA-ssDNA complex

Literature references


Editions

2006-02-25 Authored Borowiec, JA.
2006-02-25 Edited D'Eustachio, P.
Binding of ATR-ATRIP to the RPA-ssDNA complex

**Location:** Activation of ATR in response to replication stress

**Stable identifier:** R-HSA-176250

**Type:** binding

**Compartments:** nucleoplasm

ATR kinase activity is stimulated upon binding of the ATR-ATRIP complex to an RPA-ssDNA complex. ATR can subsequently phosphorylate and activate the checkpoint kinase Chk1, allowing further amplification of the checkpoint signal. The ATR and Chk1 kinases then modify a variety of factors that can lead to stabilization of stalled DNA replication forks, inhibition of origin firing, inhibition of cell cycle progression, mobilization of DNA repair factors, and induction of apoptosis. This checkpoint signaling mechanism is highly conserved in eukaryotes, and homologues of ATR and ATRIP are found in such organisms as S. cerevisiae (Mec1 and Ddc2, respectively), S. pombe (rad3 and rad26, respectively), and X. laevis (Xatr and Xatrip, respectively).

The ATR (ATM- and rad3-related) kinase is an essential checkpoint factor in human cells. In response to replication stress (i.e., stresses that cause replication fork stalling) or ultraviolet radiation, ATR becomes active and phosphorylates numerous factors involved in the checkpoint response including the checkpoint kinase Chk1. ATR is invariably associated with ATRIP (ATR-interacting protein) in human cells. Depletion of ATRIP by siRNA causes a loss of ATR protein without affecting ATR mRNA levels indicating that complex formation stabilizes the ATR protein. ATRIP is also a substrate for the ATR kinase, but modification of ATRIP does not significantly regulate the recruitment of ATR-ATRIP to sites of damage, the activation of Chk1, or the modification of p53.

While the ATR-ATRIP complex binds only poorly to RPA complexed with ssDNA lengths of 30 or 50 nt, binding is significantly enhanced in the presence of a 75 nt ssDNA molecule. Complex formation is primarily mediated by physical interaction between ATRIP and RPA. Multiple elements within the ATRIP molecule can bind to the RPA-ssDNA complex, including residues 1-107 (highest affinity), 218-390, and 390-791 (lowest affinity). Although the full-length ATRIP is unable to bind ssDNA, an internal region (108-390) can weakly bind ssDNA when present in rabbit reticulocyte lysates. ATR can bind to the ssDNA directly independent of RPA, but this binding is inhibited by ATRIP. Upon binding, the ATR kinase becomes activated and can directly phosphorylate substrates such as Rad17.

**Preceded by:** Stalling of DNA replication fork and RPA binding

**Followed by:** Activation of claspin

**Literature references**


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Recruitment of Rad17-RFC complex to DNA

**Location:** Activation of ATR in response to replication stress

**Stable identifier:** R-HSA-176101

**Type:** binding

**Compartments:** nucleoplasm

The Rad17-RFC complex is involved in an early stage of the genotoxic stress response. The major function of the protein complex is to load the Rad9-Hus1-Rad1 (9-1-1) complex onto DNA at sites of damage and/or stalled replication forks. This reaction is conceptually similar to the loading of the PCNA sliding clamp onto DNA by RFC. The association of the Rad17-RFC complex with ssDNA or gapped or primed DNA is significantly stimulated by RPA, but not by the heterologous E. coli SSB. Loading of the human 9-1-1 complex onto such DNA templates is also strongly stimulated by cognate RPA, but not yeast RPA. Although Rad17 and Rad9 are substrates of the ATR kinase activity, loading of the Rad17 and 9-1-1 complexes onto DNA occurs independent of ATR.

The Rad17-RFC complex is a heteropentamer structurally similar to RFC. The complex contains the four smaller RFC subunits (Rfc2 [p37], Rfc3 [p36], Rfc4 [p40], and Rfc5 [p38]) and the 75 kDa Rad17 subunit in place of the Rfc1 [p140] subunit. The Rad17 complex contains a weak ATPase that is slightly stimulated by primed DNA. Along with binding the 9-1-1 complex and RPA, the Rad17-RFC complex interacts with human MCM7 protein. Each of these interactions is critical for Chk1 activation.

The Rad17 subunit is conserved evolutionarily with the protein showing 49% identity at the amino acid level with the S. pombe rad17 protein. Targeted deletion of the N-terminal region of mouse Rad17 leads to embryonic lethality, strongly suggesting that human Rad17 is also essential for long-term viability.

Rad17-RFC complex associates with DNA substrates containing ssDNA regions including gapped or primed DNA in an ATP-independent reaction. Loading of the Rad9-Hus1-Rad1 (9-1-1) complex occurs preferentially on DNA substrates containing a 5' recessed end. This contrasts with the loading of PCNA by RFC which preferentially occurs on DNA with 3' recessed ends.

**Preceded by:** Stalling of DNA replication fork and RPA binding

**Followed by:** Recruitment of the Rad9-Hus1-Rad1 complex to DNA

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Recruitment of the Rad9-Hus1-Rad1 complex to DNA

**Location:** Activation of ATR in response to replication stress

**Stable identifier:** R-HSA-176264

**Type:** transition

**Compartments:** nucleoplasm

The 9-1-1 complex is a heterotrimeric ring-shaped structure that is loaded onto DNA by the Rad17-RFC complex. In vitro studies indicate that loading is preferred onto DNA substrates containing ssDNA gaps that presumably resemble structures found upon replication fork stalling and DNA polymerase/helicase uncoupling. The Rad17-RFC and 9-1-1 complexes are structurally similar to the RFC (replication factor C) clamp loader and PCNA sliding clamp, respectively, and similar mechanisms are thought to be used during loading of the 9-1-1 complex and PCNA. Upon loading, the 9-1-1 complex can recruit Chk1 onto sites of replication fork uncoupling or DNA damage.

The purified Rad17 and Rad9-Hus1-Rad1 (9-1-1) complexes can form a stable co-complex in the presence of ATP, using Rad17-Rad9 interactions. From computer modeling studies, the Rad17 subunit of the complex is also proposed to interact with the C-terminus of Rad1, p36 with the C-terminus of Hus1, and p38 with the C-terminus of Rad9. A major known function of the 9-1-1 complex is to recruit Chk1 to stalled replication forks for activation by ATR. However, the presence of the 9-1-1 complex also alters the ability of Rad17 to become phosphorylated, perhaps suggesting that 9-1-1 may regulate the recruitment of additional ATR substrates. The 9-1-1 complex has also been found to interact with base excision repair factors human DNA polymerase beta, flap endonuclease FEN1, and the S. pombe MutY homolog (SpMYH), indicating that 9-1-1 also plays a direct role in DNA repair.

**Preceded by:** Recruitment of Rad17-RFC complex to DNA

**Followed by:** Recruitment and activation of Chk1

**Literature references**


Claspin is loaded onto DNA replication origins during replication initiation. Studies in Xenopus egg extracts indicate claspin loading requires the presence of Cdc45, a factor that promotes the initial unwinding of the origin DNA in the presence of Cdk2. This step is followed by RPA binding which is a prerequisite for recruitment of PCNA and DNA polymerases alpha and delta. As RPA is not required for claspin binding, it is postulated that claspin binds at the time of initial origin unwinding but prior to the initiation of DNA synthesis. Claspin would then continue to associate with replication fork machinery where it can serve as a checkpoint sensor protein. Even though associated with the replication fork, claspin is not an essential DNA replication factor.

Studies of Xenopus claspin indicate that it can physically associate with cognate Cdc45, DNA polymerase epsilon, RPA, RFC, and Rad17-RFC on chromatin. Studies of purified human claspin indicate that it binds with high affinity to branched (or forked) DNA structures that resemble stalled replication forks. Electron microscopy of these complexes indicates that claspin binds as a ring-like structure near the branch. The protein is hypothesized to encircle the DNA at these sites.

Followed by: Activation of claspin

Literature references

Activation of claspin

Location: Activation of ATR in response to replication stress

Stable identifier: R-HSA-176298

Type: transition

Compartments: nucleoplasm

Claspin is a replication fork-associated protein important for Chk1 activation. Claspin loads onto the fork during replication origin firing and travels with the fork during DNA synthesis. Upon fork uncoupling and ATR-ATRIP binding to persistent ssDNA, the activated ATR kinase phosphorylates claspin at two primary sites. Modification increases the affinity of claspin for Chk1. Studies of human or Xenopus claspin indicate that phosphorylation of both sites is essential for significant claspin-Chk1 association. Following claspin modification by ATR, Chk1 can be transiently recruited to the stalled replication fork for subsequent phosphorylation and activation by ATR. Activation of Chk1 allows modification of additional downstream targets, thus amplifying the checkpoint signal. While much of the mechanistic information concerning claspin action has been obtained using Xenopus laevis egg extracts and Xenopus claspin, factors with similar activity have been found in various eukaryotic species including S. cerevisiae (MRC1), S. pombe (mrc1), and humans.

Activated ATR phosphorylates human claspin on two sites, threonine 916 and serine 945.

Preceded by: Loading of claspin onto DNA during replication origin firing, Binding of ATR-ATRIP to the RPA-ssDNA complex

Followed by: Recruitment and activation of Chk1

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Recruitment and activation of Chk1

Location: Activation of ATR in response to replication stress

Stable identifier: R-HSA-176116

Type: transition

Compartments: nucleoplasm

Chk1 is a checkpoint kinase activated during genotoxic stress. Like ATR, Chk1 is essential for viability in mammals. Targeted gene disruption in mice shows that loss of Chk1 causes peri-implantation embryonic lethality. Even though ATR-ATRIP not bound to ssDNA can phosphorylate Chk1, Chk1 activation is greatly enhanced when recruited to stalled replication forks by physical interaction with a modified form of claspin and the Rad9-Hus1-Rad1 sliding clamp. Activation of Chk1 occurs following phosphorylation of two sites (serine 317 and serine 345). Mutational analysis indicates that modification of both sites is essential for maximal kinase activity, while phosphorylation of only a single site causes only weak activation of Chk1. Following phosphorylation, Chk1 can diffuse away from the complex to further amplify the checkpoint signal. ATR appears to be the primary kinase activating Chk1 as conditions that activate ATR (ultraviolet irradiation or treatment with hydroxyurea) also activate Chk1. Stresses that activate ATM, e.g., ionizing irradiation, do not cause significant Chk1 activation. While the ATR and ATM pathways are distinct, there is interplay between the two. For example, double-strand DNA breaks can be processed in an ATM-dependent manner to generate structures that can cause ATR and hence Chk1 activation. The ATR and ATM pathways also have mechanistic similarities. Analogous to the Chk1 kinase existing downstream of ATR, the Chk2 checkpoint kinase is modified and activated by ATM. Although having distinct structures, Chk1 and Chk2 also have overlapping targets with some substrate sites phosphorylatable by both kinases (e.g., serine 20 of p53).

Preceded by: Recruitment of the Rad9-Hus1-Rad1 complex to DNA, Activation of claspin

Followed by: Phosphorylation of Cdc25A at Ser-123 by Chk1, Phosphorylation of Cdc25C at Ser 216 by Chk1

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Detection of DNA damage caused by ionizing radiation results in the phosphorylation of Cdc25A at Ser-123 by Chk1, inhibiting Cdc25A.

**Preceded by:** Recruitment and activation of Chk1

**Literature references**

Phosphorylation of Cdc25C at Ser 216 by Chk1

**Location:** Activation of ATR in response to replication stress

**Stable identifier:** R-HSA-75010

**Type:** transition

**Compartments:** nucleoplasm

Phosphorylation of Cdc25C at Ser 216 results in both the inhibition of Cdc25C phosphatase activity and the creation of a 14-3-3 docking site (Peng et al. 1997).

**Preceded by:** Recruitment and activation of Chk1

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