Formation of TC-NER Pre-Incision Complex

Fousteri, M., Gopinathrao, G., Hoeijmakers, JH., Orlic-Milacic, M.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of Creative Commons Attribution 4.0 International (CC BY 4.0) License. For more information see our license.
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.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 69

This document contains 1 pathway and 7 reactions (see Table of Contents)

https://www.reactome.org
Formation of TC-NER pre-incision complex is initiated when the RNA polymerase II (RNA Pol II) complex stalls at a DNA damage site. The stalling is caused by misincorporation of a ribonucleotide opposite to a damaged base (Brueckner et al. 2007). Cockayne syndrome protein B (ERCC6, CSB) binds stalled RNA Pol II and recruits Cockayne syndrome protein A (ERCC8, CSA). ERCC8 is part of an ubiquitin ligase complex that also contains DDB1, CUL4A or CUL4B and RBX1. This complex is implicated in the regulation of TC-NER progression probably by ubiquitinating one or more factors involved in this pathway, which may include RNA Pol II and ERCC6 at the later stages of repair (Bregman et al. 1996, Fousteri et al. 2006, Grosman et al. 2006). XPA is recruited to the TC-NER site through its interaction with the TFIIH complex (Furuta et al. 2002, Ziani et al. 2014). The XAB2 complex, which probably regulates the accessibility of the DNA damage site through its RNA-DNA helicase activity, binds the TC-NER site via the interaction of its XAB2 subunit with RNA Pol II, ERCC6, ERCC8 and XPA (Nakatsu et al. 2000, Sollier et al. 2014). TCEA1 (TFIIS) is a transcription elongation factor that may facilitate backtracking of the stalled RNA Pol II, enabling access of repair proteins to the DNA damage site and promotes partial digestion of the 3' protruding end of the nascent mRNA transcript by the backtracked RNA Pol II, allowing resumption of RNA synthesis after damage removal (Donahue et al. 1994). Access to DNA damage site is also facilitated by chromatin remodelers HMGN1 (recruited to the TC-NER site through RNA Pol II and ERCC8-dependent manner) and histone acetyltransferase p300 (EP300), recruited to the TC-NER site through ERCC6-dependent manner (Birger et al. 2003, Fousteri et al. 2006). UVSSA protein interacts with ubiquitinated ERCC6 and RNA Pol II, recruiting ubiquitin protease USP7 to the TC-NER site and promoting ERCC6 stabilization (Nakahazawa et al. 2012, Schwertman et al. 2012, Zhang et al. 2012, Fei and Chen 2012).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Status</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-01-29</td>
<td>Authored</td>
<td>Hoeijmakers, JH.</td>
</tr>
<tr>
<td>2004-02-02</td>
<td>Authored</td>
<td>Gopinathrao, G.</td>
</tr>
<tr>
<td>2015-06-16</td>
<td>Authored, Edited, Revised</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2015-08-03</td>
<td>Reviewed</td>
<td>Fousteri, M.</td>
</tr>
</tbody>
</table>
RNA Pol II initiates transcription from damaged DNA template

**Location:** Formation of TC-NER Pre-Incision Complex

**Stable identifier:** R-HSA-6781818

**Type:** omitted

**Compartments:** nucleoplasm

Once the transcription is initiated from a DNA template that contains an RNA polymerase II (RNA Pol II) promoter, RNA Pol II synthesizes mRNA in the presence of the elongation complex TFIIH until the damaged DNA base(s) is reached (Brueckner et al. 2007).

**Followed by:** Active RNA Pol II complex transcribes lesion-containing DNA template

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Authorship Details</th>
<th>Author/Editor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-01-29</td>
<td>Authored</td>
<td>Hoeijmakers, JH.</td>
</tr>
<tr>
<td>2004-02-02</td>
<td>Authored</td>
<td>Gopinathrao, G.</td>
</tr>
<tr>
<td>2015-06-16</td>
<td>Authored, Edited, Revised</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2015-08-03</td>
<td>Reviewed</td>
<td>Fousteri, M.</td>
</tr>
</tbody>
</table>
An active RNA polymerase II complex (RNA Pol II, POLR2) transcribes a damaged DNA template. Once damaged DNA bases, such as cyclobutane pyrimidine dimers (CPDs), enter the active site of the polymerase, RNA Pol II misincorporates a ribonucleotide into nascent mRNA, which blocks the translocation step and results in polymerase stalling. In the stalled complex, the lesion is inaccessible, while the RNA Pol II conformation is unchanged (Brueckner et al. 2007).

**Preceded by:** RNA Pol II initiates transcription from damaged DNA template

**Followed by:** ERCC6 binds stalled RNA Pol II

**Literature references**

ERCC6 binds stalled RNA Pol II

Location: Formation of TC-NER Pre-Incision Complex

Stable identifier: R-HSA-6781840

Type: binding

Compartments: nucleoplasm

Cockayne syndrome protein B (ERCC6, also known as CSB) binds RNA polymerase II complex (RNA Pol II) stalled at a DNA damage site (Fousteri et al. 2006).

Preceded by: Active RNA Pol II complex transcribes lesion-containing DNA template

Followed by: ERCC8 (CSA) binds stalled RNA Pol II

Literature references

Cockayne syndrome protein A (ERCC8, also known as CSA) is recruited to a stalled RNA polymerase II complex (RNA Pol II) at a site of DNA damage in an ERCC6 (CSB) dependent manner (Fousteri et al. 2006). ERCC8 is part of an ubiquitin ligase complex that, in addition to ERCC8, also contains DDB1, CUL4 (CUL4A or CUL4B) and RBX1 (Groisman et al. 2003). The COP9 signalosome complex prevents the ubiquitin ligase activity of the ERCC8:DDB1:CUL4:RBX1 at the early steps after DNA damage induction (Groisman et al. 2003, Fischer et al. 2011).

Preceded by: ERCC6 binds stalled RNA Pol II

Followed by: ERCC8:DDB1:CUL4:RBX1 ubiquitinates ERCC6 and RNA Pol II

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Author/Editor/Review</th>
<th>Author/Editor/Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-01-29</td>
<td>Authored</td>
<td>Gopinathrao, G., Hoeijmakers, JH.</td>
</tr>
<tr>
<td>2015-06-16</td>
<td>Authored, Edited, Revised</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2015-08-03</td>
<td>Reviewed</td>
<td>Fousteri, M.</td>
</tr>
</tbody>
</table>
ERCC8:DDB1:CUL4:RBX1 ubiquitinates ERCC6 and RNA Pol II

Location: Formation of TC-NER Pre-Incision Complex

Stable identifier: R-HSA-6781867

Type: transition

Compartments: nucleoplasm

The ubiquitin ligase complex ERCC8:DDB1:CUL4:RBX1 may ubiquitinate ERCC6 (CSB) (Groisman et al. 2006) at the later steps of TC-NER and may also be required in the ubiquitination of the RNA Pol II subunit POLR2A in response to damage (Bregman et al. 1996, Lee et al. 2002). Ubiquitination mediated by ERCC8 (CSA) containing ubiquitin ligase complex plays an important role in progression and termination of transcription-coupled nucleotide excision repair (TC-NER), although the mechanistic details are largely unknown.

Preceded by: ERCC8 (CSA) binds stalled RNA Pol II

Followed by: Assembly of the pre-incision complex in TC-NER

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015-06-16</td>
<td>Authored, Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2015-08-03</td>
<td>Reviewed</td>
<td>Fousteri, M.</td>
</tr>
</tbody>
</table>

https://www.reactome.org
In addition to ERCC6 (CSB) and the ERCC8 (CSA) ubiquitin ligase complex, several other proteins and protein complexes are loaded onto stalled RNA polymerase II (RNA Pol II) at DNA damage sites to form a pre-incision complex that operates in the transcription-coupled nucleotide excision repair (TC-NER).

XPA, which also participates in global genome nucleotide excision repair (GG-NER), is necessary for the progression of TC-NER (Furuta et al. 2002). XPA interacts with the GTF2H5 subunit of the TFIIH complex (Ziani et al. 2014). In GG-NER, XPA loading is accompanied by the release of the CAK subcomplex from TFIIH (Coin et al. 2008), but in TC-NER the CAK complex remains bound to the TC-NER site (Mourgues et al. 2013).

XAB2 protein exists in the complex with five other proteins, AQR, PRPF19, ZNF830, ISY1 and PPIE. The XAB2 complex, which is also involved in pre-mRNA splicing, loads onto stalled RNA Pol II site (Kuraoka et al. 2008) through the interaction of XAB2 with RNA Pol II, ERCC6, ERCC8 and XPA (Nakatsu et al. 2000). The AQR (aquarius) subunit of the XAB2 complex is an RNA-DNA helicase that processes R-loops. An R-loop is a structure formed by hybridization of a nascent mRNA with a DNA template. In the absence of AQR, TC-NER machinery processes R-loops into double strand breaks (Sollier et al. 2014).

TCEA1 (TFIIS) is a transcription elongation factor that facilitates partial digestion of the 3' protruding end of the nascent transcript by a stalled RNA Pol II, which is generated during the reverse translocation of RNA Pol II from the damage site, and allows the resumption of RNA synthesis once the DNA damage is removed (Donahue et al. 1994).

HMGN1, a non-histone high mobility group N nucleosome-binding protein, facilitates TC-NER probably by increasing accessibility of damaged DNA to repair machinery. HMGN1 is recruited at RNA Pol II/TC-NER sites in an ERCC8 (CSA)-dependent manner (Birger et al. 2003, Fousteri et al. 2006).

Histone acetyltransferase p300 (EP300) is recruited to stalled RNA Pol II/TC-NER complexes in an ERCC6-dependent manner, and probably acts to facilitate access of repair proteins to damaged DNA via chromatin remodeling (Fousteri et al. 2006).
UVSSA protein forms a complex with ubiquitin protease USP7. It is recruited to TC-NER sites via interaction with ubiquitinated RNA Pol II and ERCC6. The UVSSA:USP7 complex stabilizes ERCC6, preventing its proteasome-mediated degradation prior to TC-NER completion, and may de-ubiquitinate RNA Pol II after TC-NER is completed, to allow resumption of RNA synthesis (Nakazawa et al. 2012, Schwertman et al. 2012, Zhang et al. 2012, Fei and Chen 2012).

**Preceded by:** ERCC8:DDB1:CUL4:RBX1 ubiquitinates ERCC6 and RNA Pol II

**Followed by:** UVSSA:USP7 deubiquitinates ERCC6

**Literature references**


UVSSA:USP7 deubiquitinates ERCC6

Location: Formation of TC-NER Pre-Incision Complex

Stable identifier: R-HSA-6782069

Type: transition

Compartments: nucleoplasm


Preceded by: Assembly of the pre-incision complex in TC-NER

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015-06-16</td>
<td>Authored, Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2015-08-03</td>
<td>Reviewed</td>
<td>Fousteri, M.</td>
</tr>
</tbody>
</table>

https://www.reactome.org
Table of Contents

Introduction 1

Formation of TC-NER Pre-Incision Complex 2
  RNA Pol II initiates transcription from damaged DNA template 4
  Active RNA Pol II complex transcribes lesion-containing DNA template 5
  ERCC6 binds stalled RNA Pol II 6
  ERCC8 (CSA) binds stalled RNA Pol II 7
  ERCC8:DDB1:CFI:RBX1 ubiquitiates ERCC6 and RNA Pol II 8
  Assembly of the pre-incision complex in TC-NER 9
  UVSSA:USP7 deubiquitinates ERCC6 11

Table of Contents 12