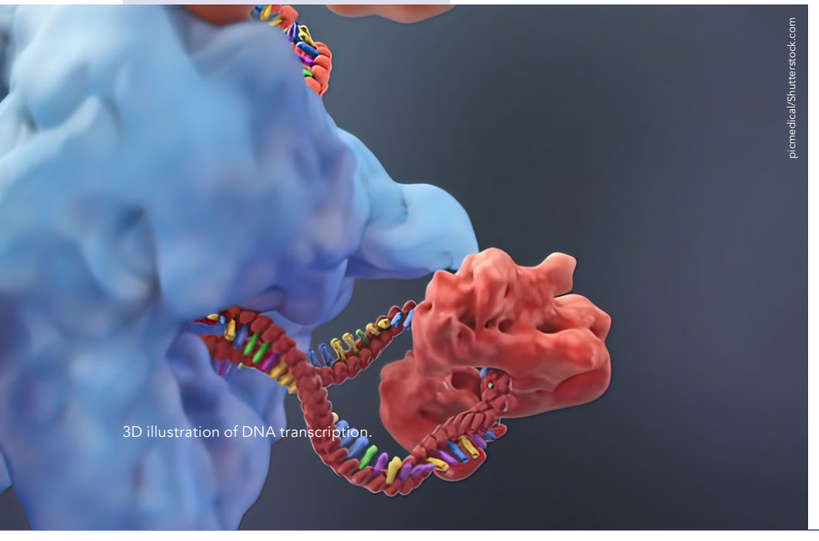
The role of BRCA1-BARD1 complex in transcription regulation

RNA polymerase II (Pol II) promoter proximal pausing in early elongation represents an important regulatory stage for proper gene expression. With years of dedicated research into the eukaryotic transcriptional regulation, Dr Heeyoun Bunch at Kyungpook National University, South Korea, is shedding light on the complex mechanisms of transcription-coupled DNA breaks and activation of damage response, which appear to be required for efficient Pol II-pause release and transcription in stress-inducible genes. Her recent work offers a novel mechanism in which the breast cancer 1 (BRCA1)-BRCA1-associated ring domain 1 (BARD1) complex is involved in immediate early gene expression by modulating the topoisomerase IIβ enzyme.

Protein synthesis is a multi-stage process. The transfer of the genetic instructions stored in DNA and needed to make polypeptides to messenger RNA (mRNA) molecules is called transcription. Transcription simply converts the genetic information to these intermediary molecules that are used for the synthesis of proteins by a complex process called translation. Overall, the production of a functional protein encoded in a gene is referred to as gene expression. Gene expression can be modulated at any stage to ensure the maintenance of the cellular processes, including developmental changes, proliferation, and cellular responses to external stimuli.

Accurate transcription is essential for preserving genome integrity and proper gene expression. The cells evolve complex pathways collectively establishing DNA damage response (DDR) signalling to respond to any genotoxic mechanisms that compromise the genomic fidelity. While various repair protein complexes are activated in different types of DNA lesions – typically the kinases including ataxia telangiectasia mutated (ATM) – ATM- and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) are activated upon the detection of DNA double strand break. These kinases phosphorylate their targets at the sites of the DNA break.

Breast cancer 1 (BRCA1) is an effector for precise DNA double strand break repair. The kinases ATM and ATR mediate the phosphorylation of BRCA1 at several residues in response to DNA damage. BRCA1 partners with BRCA1-associated ring domain 1 (BARD1) to form the BRCA1-BARD1 heterodimer with an ability to conjugate ubiquitin to protein substrates. This E3 ubiquitin ligase activity can link the protein to altered activities or destruction pathways. Though repair mechanisms are activated upon generation of DNA lesions, DNA breaks might be unrepairable, the genome is mutated, and the cell undergoes programmed cell death if the damage is too severe.



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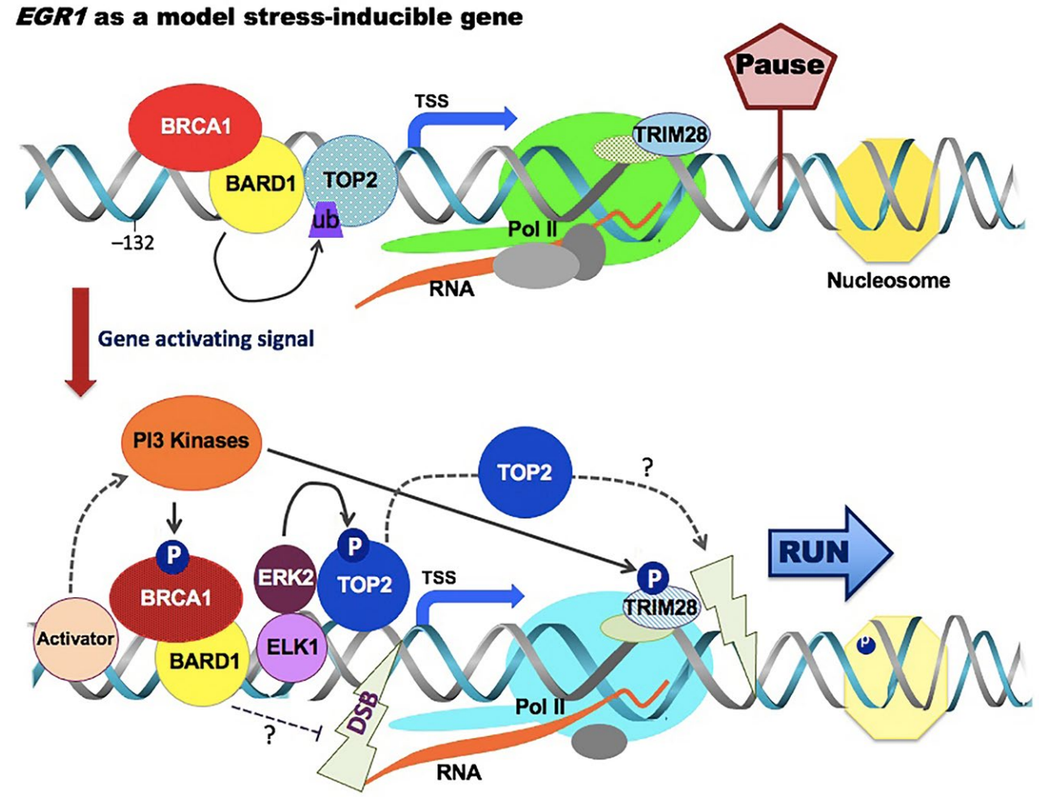
3D illustration of DNA transcription.

RNA POLYMERASE II PROMOTER-PROXIMAL PAUSING FOR PROMPT AND DECISIVE CELL REGULATION

In eukaryotes, there are three RNA polymerases catalysing the transcription from DNA to RNA. Among them, RNA polymerase II (Pol II) targets all protein-coding genes and many noncoding RNAs. Transcription initiation requires the association of DNA-binding transcription factors (TFs) with their target motifs and the employment of Pol II and general TFs to position Pol II correctly at the promoter region, where transcription begins. For years, initiation, elongation along the full length of the gene and termination were considered the major steps of transcription that are tightly controlled by various protein and nucleic acid factors. Intriguingly, gene expression can also be regulated in early elongation prior to processive elongation, where Pol II pauses at around nucleotides +25–100 relative to the transcription start site (TSS), awaiting further signals to induce elongation. Only, the controlled release of paused Pol II produces a functional full-length mRNA. This phenomenon, namely Pol II promoter-proximal pausing, represents a major checkpoint for proper gene expression and is exhibited by approximately over 30% of coding genes and more than 70% of developmental or stimulus-inducible genes.



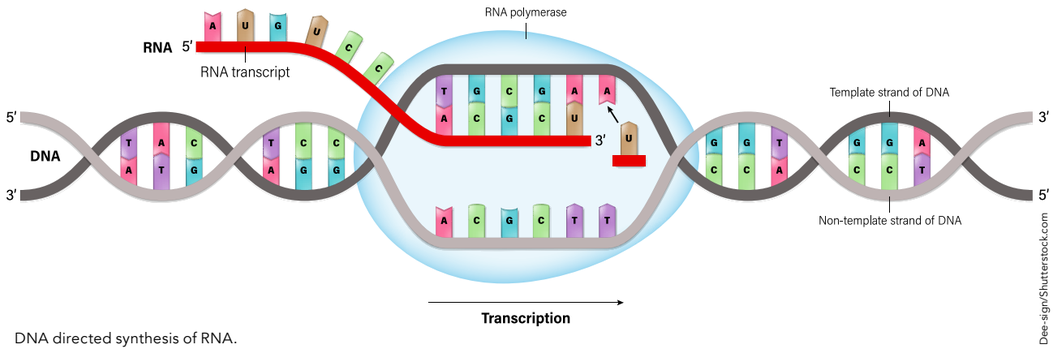
Stress-inducible gene expression.

A NOVEL FINDING

Despite decades of research since its discovery in the late 1980s, the mechanisms underlying stable Pol II pausing and release aren't fully understood. Back in 2014, Dr Heeyoun Bunch and her colleagues at the Beth Israel Deaconess Medical Center and Harvard Medical School identified TRIM28, a TF and DDR protein, as a modulator of Pol II pausing and transcriptional elongation. The researchers conducted the initial studies on the human heat shock protein 70 (HSP70) gene and demonstrated that the above-mentioned processes involved the phosphorylation of TRIM28 by the prominent DDR kinases ATM and DNA-PK. Moreover, by performing genome-wide analysis, they proposed a global function for TRIM28 in controlling Pol II pausing and pause release in mammalian genes.

Accurate transcription is essential for preserving genome integrity and proper gene expression.

The findings on TRIM28 prompted Bunch and colleagues to investigate a potential involvement of DNA breaks and DDR signalling in Pol II pause release and transcriptional activation. Their research in 2015 has shed new light on the topic and presented the coupling between DDR signalling and transcriptional elongation in stimulus-inducible protein coding genes as a novel mechanism for regulation of gene expression. Importantly, the researchers proposed that the enzyme topoisomerase IIβ (TOP2B) mediates DNA double strand breaks and alters the topologic states of DNA for Pol II pause release and subsequent transcriptional elongation. TOP enzymes are responsible for the formation of transient physiological DNA breaks during normal cellular processes. They control DNA topology,



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DNA directed synthesis of RNA.

and by binding to DNA and cutting the phosphate backbone, they also allow RNA polymerase action during transcription. Bunch and her colleagues raised great interest by presenting the involvement of TOP2B in transcriptional activation-coupled DNA breaks and repair signalling.

REPOSITIONING OF BRCA1-BARD1 COMPLEX

After establishing a link between DDR signalling and transcriptional elongation, the group further explored the role of DDR signalling in stress-inducible gene expression. To do that, they evaluated the function of the BRCA1-BARD1 complex in Pol II elongation of several human immediate early genes. These genes represent transcription factors and proto-oncogenes that are promptly activated in response to appropriate signals and stimuli such as cell proliferation signals. The transcription of these genes involves Pol II promoter-proximal pausing and stimulus-induced pause release.

The extensive experimentation, carefully designed to uncover the mechanisms of transcriptional regulation, indicated a new function for the BRCA1-BARD1 complex involved in DDR signalling through modulating the affinity of TOP2B to DNA. First, the researchers measured the ATM and ATR-induced phosphorylation of BRCA1 and demonstrated that BRCA1 is activated in response to DNA damage with phosphorylation during transcriptional elongation of human immediate early genes JUN, FOS, MYC and early growth response factor 1 (EGR1). BRCA1 in heterodimer form with BARD1 can bind near the TSS of these genes for modulating their transcription. Based on their findings, Bunch and her colleagues proposed a model involving the resting and active states of transcription: during the resting stage, Pol II is paused in the promoter-proximal site in human immediate early genes. The BRCA1-BARD1 complex physically interacts with TOP2B and drives the ubiquitination of the enzyme by functioning as an E3 ligase. This ubiquitination leads to a tight association of the highly stable enzyme with TSS. On the other hand, when ATM/ATR phosphorylates BRCA1 during activation of transcription, the opposite takes place: BRCA1 phosphorylation changes the aforementioned physical interaction and alters/reduces the TOP2B ubiquitination, which in turn will decrease the affinity of TOP2B to the TSS of the transcriptionally active gene. Overall, depending on its phosphorylation status, the DDR signalling complex BRCA1-BARD1 modulates transcription elongation by interacting with TOP2B.

Bunch's work offers new insights into the early transcriptional regulation of stimulus-induced genes by assigning a novel role to the DDR signalling complex BRCA1-BARD1.

The BRCA1-BARD1 complex is an important tumour suppressor.

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In their latest work, Bunch and colleagues presented the BRCA1-BARD1 complex-mediated TOP2B regulation for modulation of stimulus-induced gene expression. They demonstrated not only the involvement of a DDR signalling molecules in early transcriptional regulation, but they also uncovered the mechanisms by which the BRCA1-BARD1 complex functions as an E3 ligase for altering the stability of TOP2B and its affinity to DNA during Pol II pause and pause release. Bunch's work raises great attention by offering new insight into the early transcriptional regulation of stimulus-induced genes by assigning a novel role to the DDR signalling complex BRCA1-BARD1.

Behind the Research

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Research Objectives

Dr Bunch reported a novel function of the breast cancer 1 (BRCA1)-BRCA1-associated ring domain 1 (BARD1) complex in transcriptional regulation.

Detail

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Bio
Heeyoun Bunch acquired a PhD in microbiology at Cornell University (2009) and did her postdoctoral research at the University of Colorado (2010–2011), Harvard Medical School (2012–2016), and the University of Texas Southwestern Medical Center (2016–2017).

Funding
- National Research Foundation of the Republic of Korea

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- Matthew J Schellenberg (Mayo Clinic, USA)
- Benjamin P C Chen (University of Texas Southwestern Medical Center, USA)

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Personal Response

What are the possible real-world applications?

Life depends on continuous gene expression which allows the cells to differentiate, proliferate, grow, and eventually die. Humans are composed of more than 30 trillion cells, originated and divided from a single cell. Understanding the secrets of how genes are regulated for expression is critical to comprehend cellular growth, ageing, and death.

The BRCA1-BARD1 complex is an important tumour suppressor, whose function is critical for precise DNA repair. Our study identified its novel role in transcriptional regulation: the complex is necessary for proper immediate early gene expression and yet appears to take distinctive roles depending on its modification (phosphorylation in our finding) status. In addition, the regulation of TOP2B binding to DNA and catalysis has been poorly understood. We found that the BRCA1-BARD1 complex regulates TOP2B-DNA binding by ubiquitinating TOP2B, which is an important step forward in understanding TOP2B regulation in transcription.

How important are these mechanisms for concepts like ageing or evolution?

DNA break associated with transcription can be deleterious, resulting in DNA mutations and genomic instability, which contributes to or accelerates ageing. The irony is that gene expression is the most essential cellular process that must occur, which may explain, at least in part, why we age and eventually die. I believe that understanding how the cells cope with the DNA break associated with transcription will help us to understand the fundamental process and nature of genomic instability and to find a way to decelerate it.

Where are we now and what comes next in this research area?

We are currently investigating TOP2B regulation during transcriptional activation. To gain insights into the big picture, we need to gather multiple pieces of information and results from examining an array of questions at different angles… it's like a large puzzle with many pieces. For each piece, we ask a specific question, install sound hypotheses, and perform the analyses using model genes and diverse techniques. Our research will continuously focus on and reveal the regulation of TOP2B and the repair of TOP2-mediated DNA break during transcription.