Supertitle: Innate immunity

Transcription stress causes an inflammatory response *via* release of IL-1α

Thomas DJ Walker[1], Jessica P Morris[1] and Leonie Unterholzner[1*]

1. Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, LA1 4YQ, Lancaster, UK

* Corresponding author, l.unterholzner@lancaster.ac.uk

DNA damage can be sensed as a danger signal by the innate immune system. Bournique *et al.* show that the transcription stress caused by DNA lesions can also initiate inflammation by causing the direct release of cytokine IL-1α which then drives NF-κB activation in neighbouring cells.

Damaged DNA is associated with genome instability and plays an important role in tumorigenesis. Upon detection of DNA damage, cells initiate a DNA damage response (DDR) which arrests the cell cycle and promotes DNA repair. Alongside the activation of cell cycle checkpoints and DNA repair, DNA damage also acts as a danger signal to activate an innate immune response. Reporting in *Nature Structural and Molecular Biology*, Bournique *et al*.[1] identify a novel mechanism of innate immune activation following DNA lesions that cause transcription stress. The authors find that transcription stress causes the release of the cytokine interleukin 1α (IL-1α), which can signal to cells in the microenvironment without relying on innate immune gene expression from the damaged cell. The authors show that this signalling pathway is independent of ataxia telangiectasia mutated (ATM)-mediated sensing of DNA double strand breaks (DSB), and that both pathways operate in parallel to induce NF-κB activation following DNA damage. This work provides further insight into the molecular mechanisms linking DNA damage and innate immunity.

Cells can sense infection and injury, by recognising pathogen-associated molecular patterns (PAMPs) during infection, and damage-associated molecular patterns (DAMPs) derived from modified or mis-localised endogenous molecules. DNA damage can result in the generation of DAMPs, but this may depend on the type of DNA lesion[2]. Several mechanisms that link DNA damage to innate immune activation have been identified

(Fig. 1). Following double strand DNA breaks (DSBs), DNA fragments may be released to the cytoplasm where they are detected by the DNA sensor cGAS (cyclic GMP-AMP synthase) and its adaptor protein STING (STimulator of Interferon Genes), which then induce the production of type-I interferons[3]. This response occurs several days after the initial DNA damaging event, possibly involving the formation of micronuclei or chromosome bridges during mitosis, even though the precise nature of the DAMPs that are detected in this context remains under debate[4–6]. DNA damage can also trigger an acute pro-inflammatory response within hours of the initial event, which is independent of cGAS. Nuclear DSBs are directly detected by the DNA damage repair kinase ATM which activates cytosolic TRAF6, an E3 ubiquitin ligase[7,8]. Under these conditions, TRAF6 can ubiquitylate STING to form an alternative DNA-damage induced signalling complex which promotes the activation and nuclear translocation of NF-κB[8]. NF- κB activation ultimately promotes the expression of pro-inflammatory cytokines.

The disruption of normal cellular functions has also recently emerged as a danger signal for the innate immune system. Bournique et al. used high content imaging to track the translocation of the NF-κB subunit p65 from the cytoplasm to the nucleus in cells, following the induction of DNA damage and/or transcription stress[1]. This allows the monitoring of cell cycle stage (by DNA content), the cellular response to DNA damage levels (γH2A.X), and NF-κB p65 activation (p65 nuclear translocation) simultaneously at the single cell level. This approach provides greater granularity into the activation of innate immune signalling cascades that may otherwise not be picked up through bulk-population analysis which conflates disparate signals from asynchronous cell cycle stages or masks inter-cell response variances.

The authors uncover two distinct modes of NF-κB p65 activation in different cell cycle stages that are dependent on DNA lesion type. Following treatment with a range of genotoxic agents that induce DSBs, rapid p65 activation was observed solely during S phase when cells are replicating DNA, and occurred through the ATM-dependent activation of TRAF6. Alternatively, when cells were treated with UV light or compounds that block transcription, such as actinomycin D, p65 activation was observed across the entire cell cycle and without the requirement for ATM or STING. Cells undergoing transcriptional stress activated NF-κB p65 indirectly, through the release of IL-1α from chromatin and its secretion into the extracellular space, followed by autocrine / paracrine signalling via the IL-1 receptor (IL-1R1). IL-1R1 signalling then elicits the IRAK1- and TRAF6-mediated activation of NF-κB p65, which accumulates in the nucleus gradually over several hours following transcription blockade. These findings demonstrate that cells initiate rapid activation of pro-inflammatory immune signalling through two distinct mechanisms involving either ATM or IL1α – IL-1R1 – IRAK1, both of which converge at TRAF6-mediated activation of NF-κB p65 (Fig. 2).

Of interest, the two pathways do not appear mutually exclusive, and the specific ATM and/or IRAK1 pathway route to NF-κB p65 translocation depends on DNA lesion type and whether transcriptional stress is incurred, rather than being a consequence of regulation by cell-cycle signalling. For example, high doses of the topoisomerase I (TOP1) inhibitor camptothecin trigger p65 nuclear translocation during S phase through both ATM-dependent and IRAK1-dependent routes, the former a consequence of TOP1-cleaved complex-derived DSB formation, the latter due to TOP1-mediated transcription blockage. The IRAK1-mediated response to transcription blockage continues to operate during G1 and G2 phases in an ATM-independent fashion. Induction of transcription stress through UV irradiation or treatment with RNA synthesis inhibitors also caused p65 activation throughout the cell cycle. These experiments show that transcription blockade is sensed as a stress-associated molecular pattern (SAMP) which causes a transient inflammatory response that is relieved following resumption of normal transcription.

A key feature of the innate immune response to transcription stress is that the initial release of IL-1α can be induced in the absence of a functional gene expression response, and thus bypasses blocks in transcription or translation. IL-1α is often present in the nucleus, and is released as alarmin during cell damage and necrosis[9]. Nuclear IL-1α has been shown to be acetylated at K82, and its de-acetylation following genotoxic stress causes its release from the nucleus and secretion as an active cytokine[10]. Bournique et al. determined through mutagenesis and the use of histone deacetylase (HDAC) inhibitors that IL-1α release following transcription stress is also dependent on deacetylation at K82[1]. The authors show that HDAC involvement is limited to initial IL-1α release, since exogenously introduced IL-1α in the presence of HDAC inhibition restored the IRAK1- p65 activation axis.

The release of IL-1α may be relevant for the initiation of inflammatory responses in the tumour microenvironment after treatment with genotoxic chemotherapies that cause transcription stress. Bournique *et al*. found that IL-1α and IL-1R1 are highly up- or down-regulated in many cancer-derived cell lines, with cells from head and neck, bladder and urinary tract cancers showing highest expression of both[1]. Of note, Il-1α-expressing cells were able to signal *in trans* to cause NF-κB p65 activation in neighbouring cells. The innate immune signalling cascades that lead to the secretion of cytokines and chemokines in the tumour micro-environment have clinical relevance for the effectiveness of both genotoxic treatments and immunotherapies[11], and may hold promise for the development of novel combination therapy approaches in the future. How the IL-1α - IRAK1 - p65 signalling axis can be exploited (or accommodated) during current and emerging therapies warrants further investigation.

Through identifying transcription stress as a danger signal for an inflammatory response, this study adds to our current understanding about the triggers that activate

the innate immune system. While innate immune sensing mechanisms were initially identified in the context of PAMPs derived from infectious agents, it has since become clear that PAMPs are often effectively shielded by pathogens that are well-adapted to their host defences, and that the presence of an infectious agent can also be sensed by virtue of its effects on a cell. This may be through the detection of virulence activities of intracellular pathogens as effector-triggered immunity[12], or alternatively through the detection of damaged endogenous molecules as DAMPs[13]. Recently, it has become clear that even transient cell stress – which does not necessarily result in damage – can act as a danger signal for the innate immune system. The findings by Bournique et al. add further evidence to this notion. Thus, transcription stress may be sensed as stress-associated molecular pattern (SAMP), analogous to sensing of ribotoxic stress – the collision of ribosomes due to translation block - which has been shown to activate the NLRP1 inflammasome[14,15]. Like transcription stress, ribotoxic stress can occur as a consequence of UV irradiation[15], but causes inflammation *via* IL-1β release and cell death by pyroptosis. Together with the data from Bournique *et al*., it can be assumed that the same initial insult can give rise to multiple parallel innate immune signalling cascades, dependent on lesion type, cell cycle stage and cell identity in terms of expression of PRRs and signalling factors. Given that the primary driver during the evolution of our innate immune system was the response to infection, it is plausible that the disruption of normal cell function is a key tell-tale sign of infection, in a scenario where cellular processes are blocked or diverted to serve an intracellular pathogen. It remains to be investigated precisely which molecular features are detected as SAMP during transcription stress, and to what extent the sensing of transcription stress contributes to the innate immune response to infection and injury.

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Competing interests

The authors declare no competing interests.

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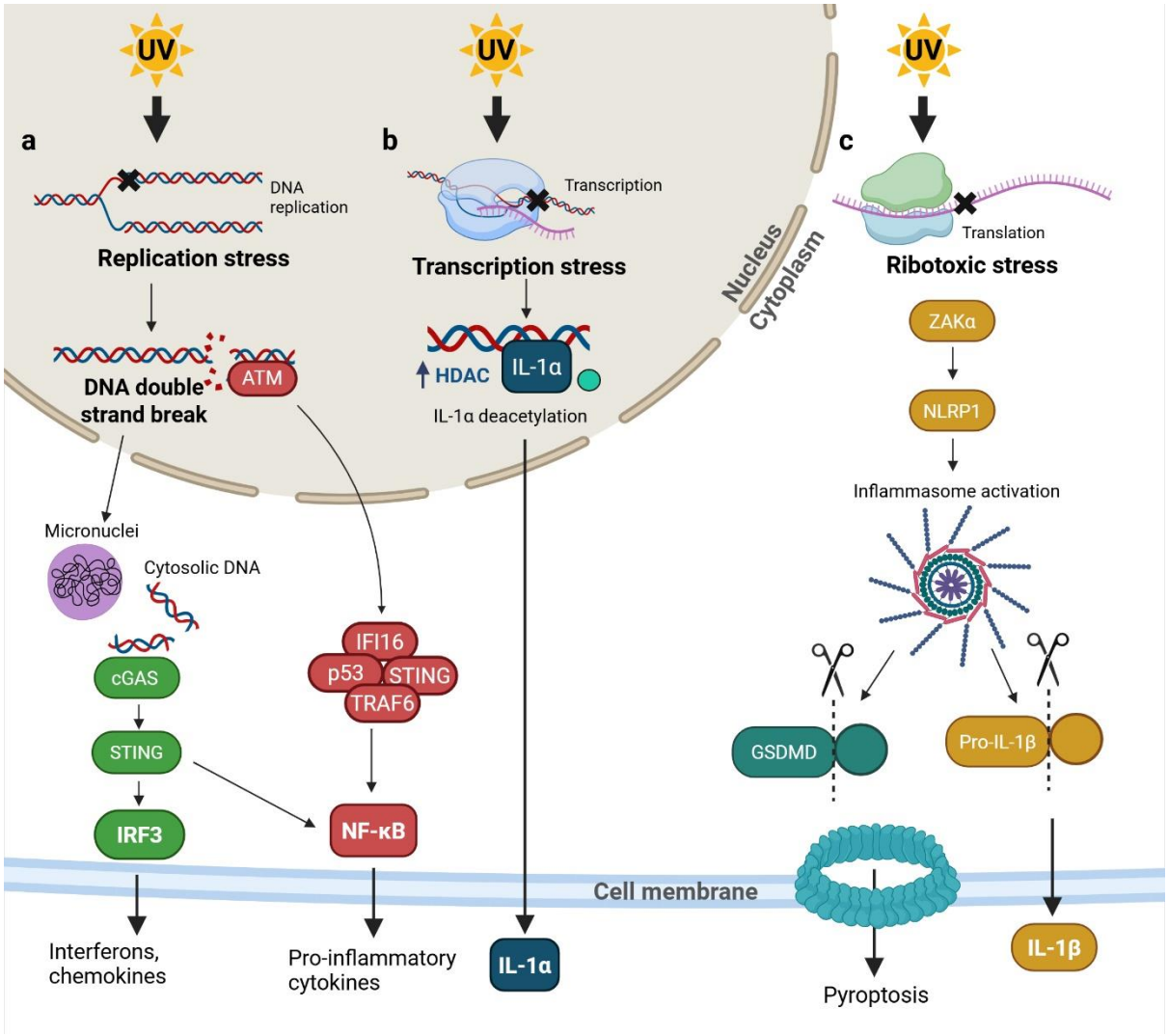

Fig. 1 Innate immune signalling pathways induced by ultraviolet (UV) radiation.

a, DNA lesions caused by UV radiation can cause replication stress and DNA double strand breaks (DSBs). Micronuclei and cytosolic DNA release can be detected by cyclic GMP-AMP synthase (cGAS) which signals through stimulator of interferon genes (STING) to activate interferon regulatory factor 3 (IRF3). This induces the expression of interferons and cytokines which are then released from the cell. Alternatively, DSBs can be directly detected by ataxia telangiectasia mutated (ATM) which signals through p53, interferon-γ-inducible factor 16 (IFI16), STING, and tumour necrosis factor receptor associated factor 6 (TRAF6) to activate nuclear factor κB (NF- κB). NF- κB activation promotes the induction of pro-inflammatory cytokines. **b**, UV radiation can induce transcriptional stress which promotes the histone deacetylase (HDAC)-mediated deacetylation of chromatin bound interleukin 1α (IL-1α) which is then released from the cell. **c**, Stalled or collided ribosomes are detected by ZAKα which activates the NLRP1 inflammasome. This promotes the proteolytic cleavage of pro-interleukin-1β (IL-1β) to IL-1β which is then secreted from the cell. Additionally, the inflammasome can promote Gasdermin D (GSDMD) pore formation in the cell membrane which leads to pyroptosis.

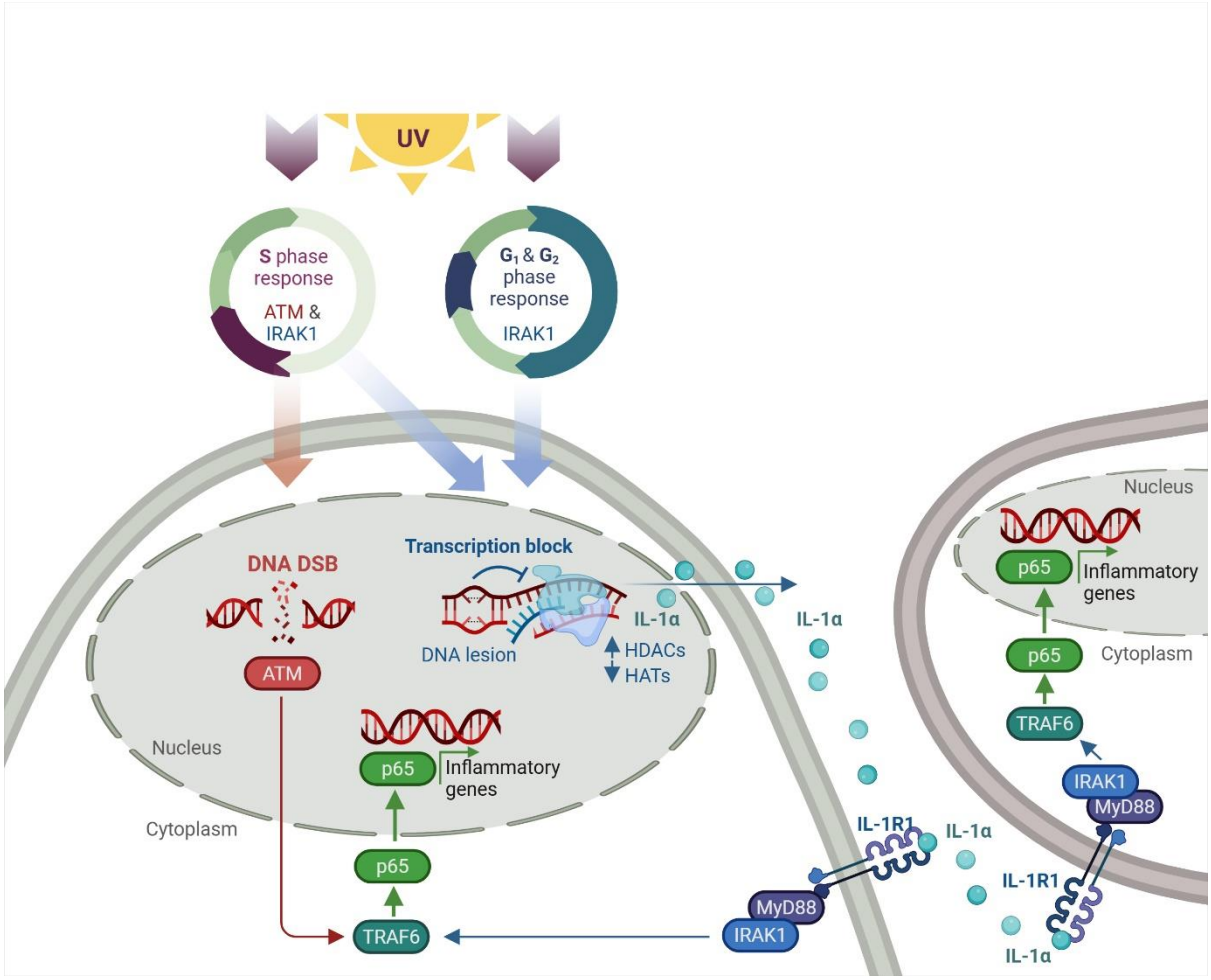

Fig. 2: Two cell cycle phase-dependent routes to NF-κB p65 activation following ultraviolet (UV) radiation exposure.

DNA double strand break (DSB) events following UV genomic assault are coordinated within cell cycle S phase by ATM during the DNA damage response (left, in red). ATM translocates to the cytoplasm to activate TRAF6. Additionally, UV-derived DNA lesions cause RNA polymerase transcription block during G1, S, and G2 cell cycle phases (right, in blue). Blocked mRNA transcription releases chromatin-associated IL-1α in a histone deacetylase (HDAC) dependent fashion. Secreted IL-1α signals extracellularly via IL-1R1 receptors in an autocrine and paracrine manner. IL-1R1 activates MyD88 and IRAK1 to enable the activation of TRAF6. Following ATM and IRAK1 pathway convergence, activated TRAF6 signals to release cytoplasmic sequestration of p65. p65 translocates to the nucleus to initiate inflammatory gene expression. In contrast to the ATM-governed pathway response to DSBs, the IL-1α – IL1-R1 – IRAK1 pathway permits cells that are unable to initiate a transcriptional response themselves to elicit a p65-dependent inflammatory response in neighbouring cells.

ATM: Ataxia-telangiectasia mutated; IL-1α: Interleukin-1 α; IL-1R1: Interleukin 1 receptor type I; MyD88: Myeloid differentiation primary response 88; IRAK1: Interleukin-1 receptor-associated kinase 1. TRAF6: TNF (tumour necrosis factor) receptor associated factor 6; p65: Nuclear Factor-κB p65 subunit; HATs: Histone acetyltransferases, HDACs: Histone deacetylases.