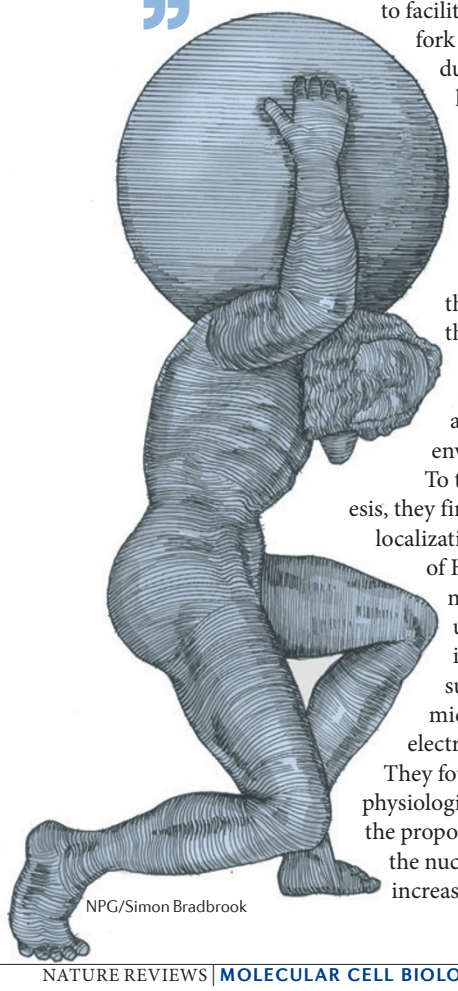
NUCLEAR ENVELOPE

ATR senses mechanical stress

> chromatin condensation that occurs during prophase generates a mechanical stress that leads to the activation of ATR



NPG/Simon Bradbrook

ATR is an essential checkpoint kinase that regulates several pathways of the DNA damage response (DDR). Now in *Cell*, Bartek, Foiani and colleagues report that mechanical stress at the nuclear envelope activates ATR independently of DDR signalling in mammalian cells and that this ATR-mediated mechanical response is important for the maintenance of genome integrity.

Previous experiments in yeast have shown that the Mec1 pathway (Mec1 is the yeast counterpart of ATR) promotes the detachment of chromatin from the nuclear envelope to facilitate replication fork progression during S phase, but how ATR senses that the detachment must occur when forks approach is not known. The authors of this study reasoned that ATR might respond to mechanical forces at the nuclear envelope.

To test this hypothesis, they first analysed ATR localization in the nuclei of HeLa cells and mouse fibroblasts using structured illumination super-resolution microscopy and electron microscopy. They found that under physiological conditions, the proportion of ATR at the nuclear envelope increased from ~10% during interphase to ~20% during prophase (the remaining nuclear ATR was in the nucleoplasm). Two other checkpoint factors, ATR-interacting protein (ATRIP) and active, phosphorylated CHK1 (pCHK1), were also located at the nuclear envelope during prophase. Moreover, whereas ATR relocation to the nuclear envelope was independent of ATR kinase activity, this was required for the prophase-specific phosphorylation and nuclear envelope localization of CHK1. Accumulation of these factors at the nuclear envelope also occurred in response to induced replication stress. Taken together, this suggests that mechanical stress that arises from chromatin condensation in prophase or during DNA replication may lead to the recruitment of ATR, and subsequently of CHK1, to the nuclear envelope and the activation of these factors there.

Next, the authors used several methods to induce mechanical stress at the nuclear envelope to directly test whether it triggers the ATR response. In hypertonic conditions, which cause ruffling of the nuclear envelope, nuclear shrinking and chromatin condensation, ATR very rapidly accumulated at the nuclear envelope, as did pCHK1 and ATRIP, in an ATR-dependent manner. Importantly, relocation and activation of ATR were independent of DNA damage and of factors that are known to activate ATR in response to DNA damage. Similarly, a patch-clamp automatized platform that stretched cells by pulling them from opposite poles resulted in chromatin condensation and the rapid redistribution of ATR to perinuclear regions. Use of a compressive-load system that deforms cells by exerting forces at a physiological range showed that the ATR response to mechanical stress occurs within this range. Moreover, these experiments showed that ATR relocalization and activation is reversible and parallels chromatin compaction and decompaction (when the mechanical stress is removed).

Finally, the authors analysed the kinetics of chromatin condensation and nuclear envelope breakdown (NEBD), which occurs early in mitosis, with or without active ATR. Both pharmacological inhibition of ATR and *ATR* gene mutations caused a delay in the onset of chromatin condensation and completion, as well as a delay in NEBD and in the detachment of perinuclear lamin A from condensed chromatin. Thus, ATR seems to function in coordinating nuclear envelope and chromatin dynamics in response to mechanical stimulations.

This study indicates that the chromatin condensation that occurs during prophase generates a mechanical stress that leads to the activation of ATR at the nuclear membrane, and that this is important for correct chromatin condensation and chromatin detachment from the nuclear membrane during cell cycle progression. It thus uncovers an ATR pathway that is distinct from the DDR and prompts further studies to elucidate the mechanisms underlying ATR localization and activation following mechanical stress.

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ORIGINAL RESEARCH PAPER Kumar, A. *et al.* ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress. *Cell* **158**, 633–646 (2014)