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Regulation of Spire1 by Phosphoinositides and PARP in DNA Damage Response

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Abstract: Phosphoinositides (PPIs) plays an essential role in regulating nuclear actin polymerization upon DNA damage. Spire1, an actin nucleator, was reported to be required for actin filament formation and DNA repair. Interestingly, Spire1 harbors a putative PPI-binding FYVE domain, which was previously shown to swiftly enrich at DNA lesions. Here, I investigated whether PPIs and PPI-related enzymes regulates Spire1-FYVE accumulation at laser-induced DNA breaks. Upon DNA damage, nuclear PPIs [PtdIns(4)P and PtdIns(4,5)P2] co-localized with Spire1-FYVE, which was repressed by inositol polyphosphate multikinase (IPMK) inhibition. Furthermore, suppressed PARP activity attenuated the accumulation of Spire1-FYVE and PPIs at DNA damage sites. Together, my results establish a potential regulatory arm of nuclear actin via the PARP/IPMK/PPI axis in response to DNA damage.

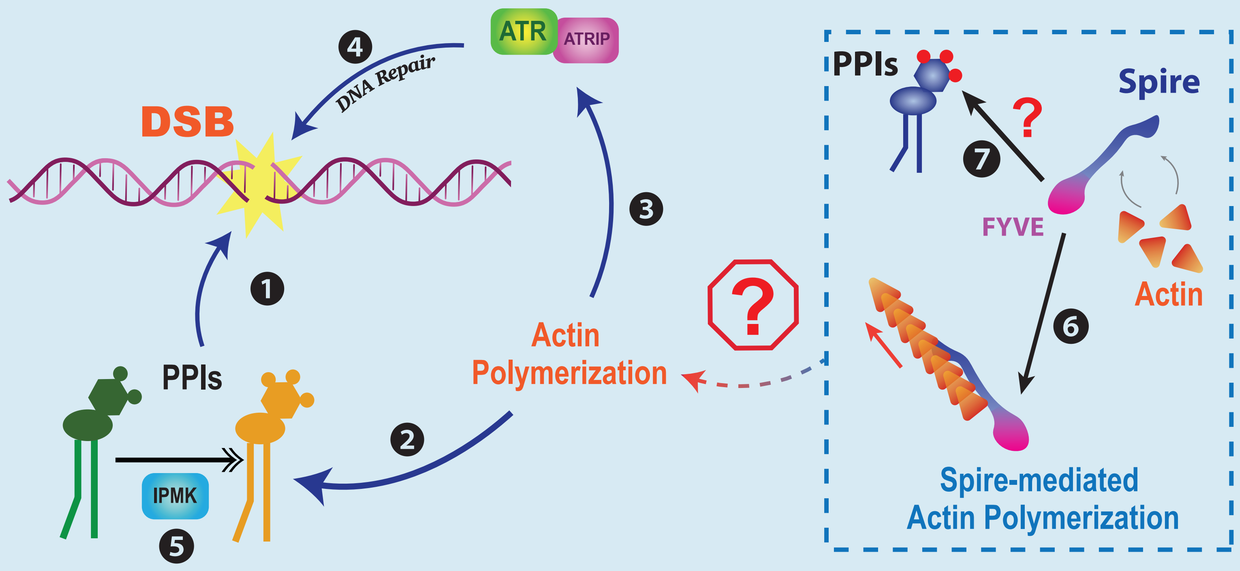
Introduction

DNA repair via actin and PPIs

Actin polymerization actively participates in various **DNA damage response (DDR)** processes, including chromatin modulation, DNA break translocation, and repair factor recruitment. Recent findings revealed ❶ **poly-phosphoinositides (PPIs)** enriched at double-strand breaks (DSBs) ❷ to stimulate actin filament formation ❸ and ATR signaling ❹ to facilitate DNA repair. Further, ❺ functions of PPIs in DDR are determined by the activity of **inositol polyphosphate multikinase (IPMK)**, a nuclear kinase converting PtdIns(4,5)P2 to PIP3. However, the molecular basis of how PPIs regulate nuclear actin remains largely enigmatic.

Spire1 – a potential link

Spire1 belongs to the **Spire actin nucleator** family, which ❻ initiates the assembly of monomeric actin into filamentous polymers. Previous studies illustrated Spire1/2 were required for DDR-directed actin polymerization, and Spire1 was also identified as a substrate of DYRK1B, a DDR kinase. Further, our lab demonstrated the accumulation of full-length Spire1 at laser-induced DSBs. Intriguingly, Spire1 harbors an **FYVE domain with putative PPI-binding capability**, ❼ indicating a **potential Spire1-PPI interaction**.

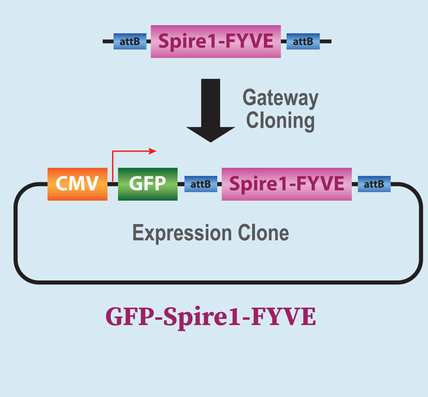


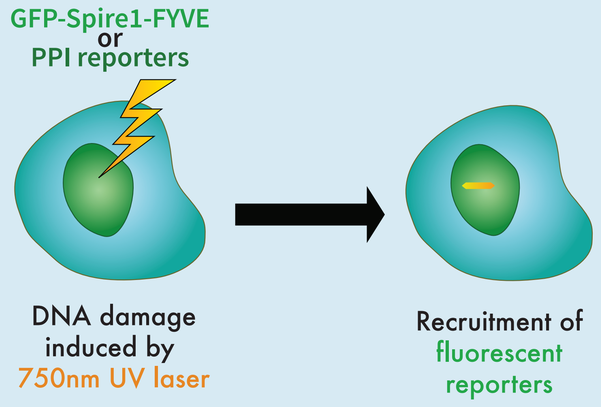
Research Aims

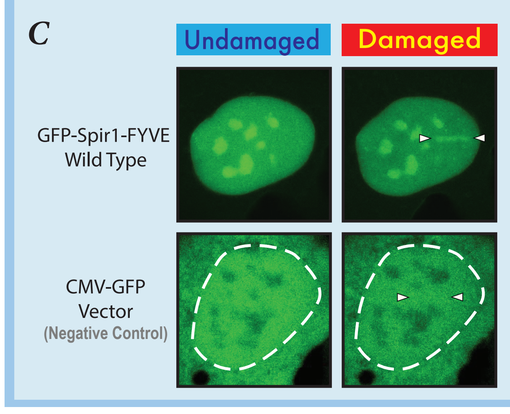
1. To illustrate DSB-accumulation of Spire1-FYVE
2. To investigate roles of PPIs and IPMK in the recruitment of Spire1-FYVE
3. To examine potential upstream regulation of PPIs (e.g., by PARP)

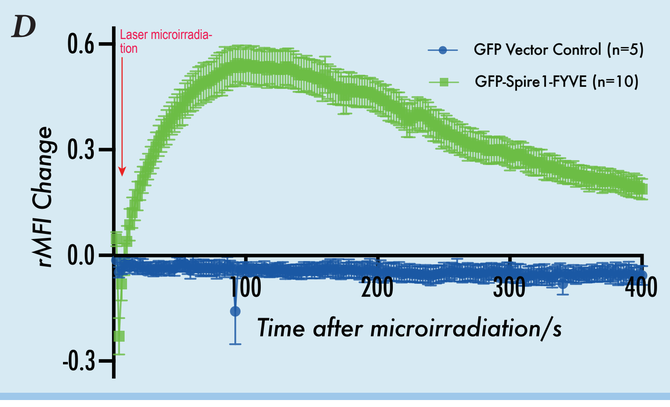
Methods & Results

(1) Accumulation of GFP-Spire1-FYVE at laser-irradiated DNA breaks.



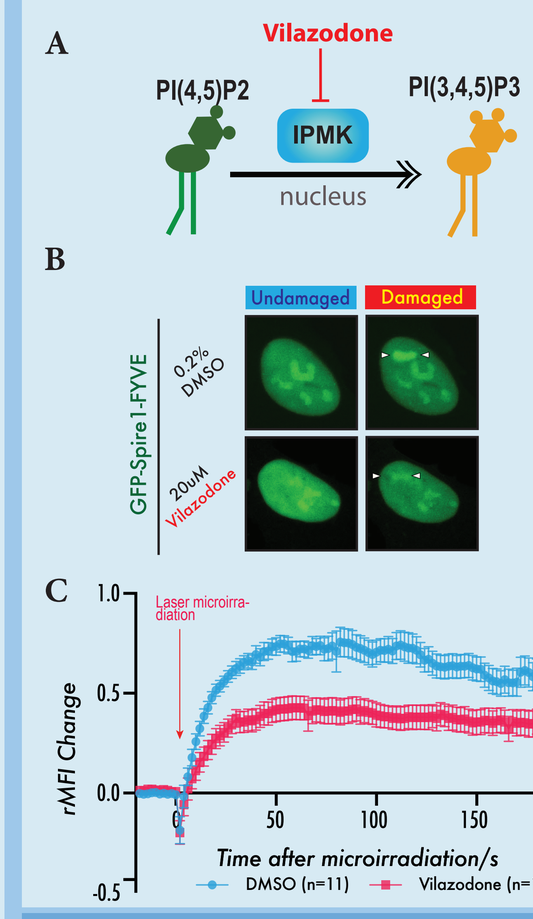




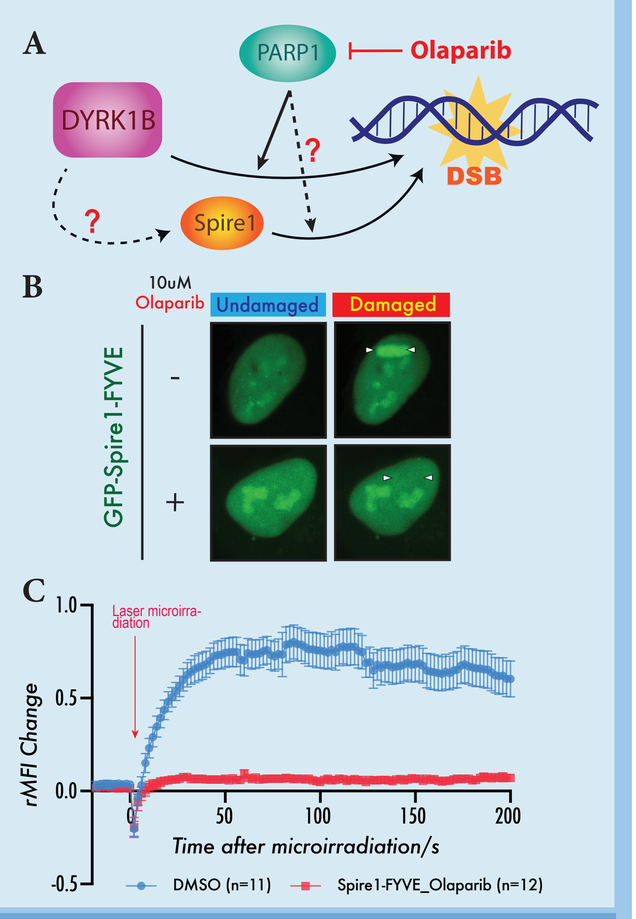


Methods & Results

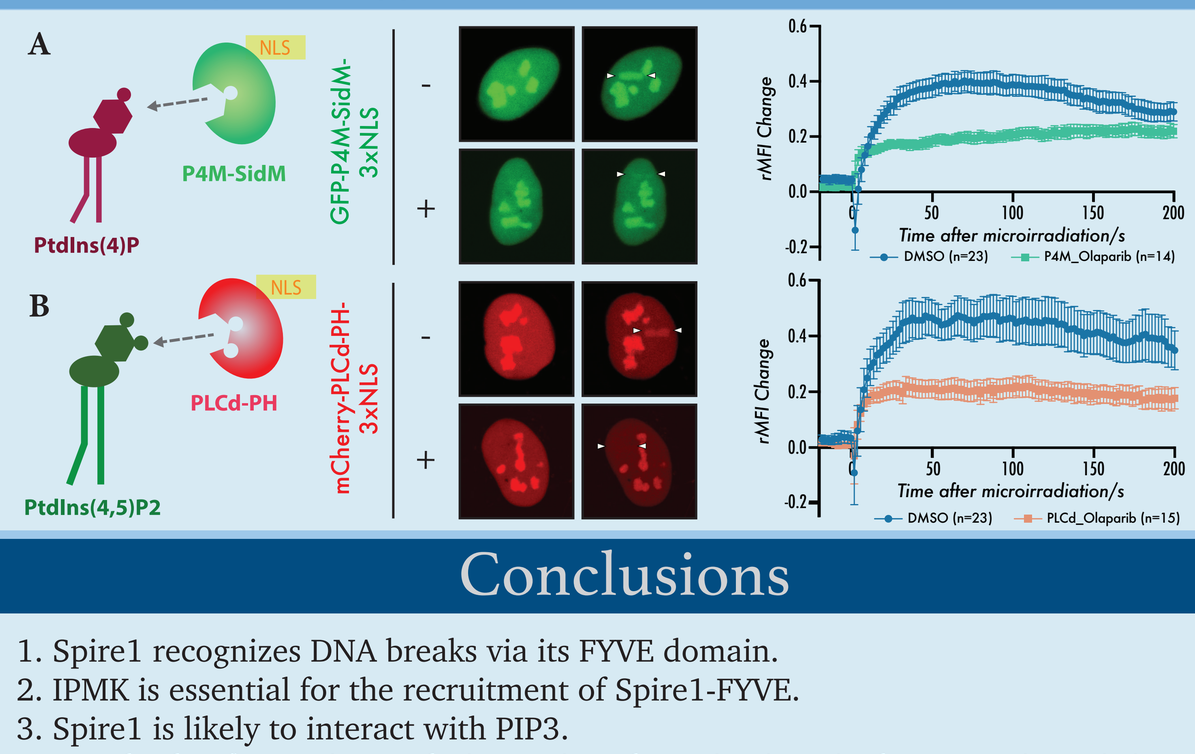
(2) IPMK inhibition attenuated Spire1-FYVE accumulation at DSB.



(3) Inhibiting PARP suppressed recruitments of Spire1-FYVE.



(4) Inhibiting PARP suppressed PPIs enrichment att DSB.



Conclusions

1. Spire1 recognizes DNA breaks via its FYVE domain.
2. IPMK is essential for the recruitment of Spire1-FYVE.
3. Spire1 is likely to interact with PIP3.
4. Novel role of PARP in regulating actin polymerization via the PPI/Spire axis in DDR.

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