Poly (ADP-ribose)

Samuel Nalin, Corbin Hopper

MPRI Biochemical Programming

March 6, 2020

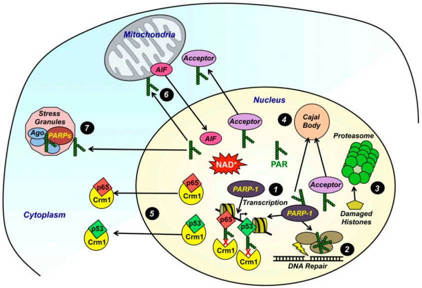
Biological Basis of PAR

PAR

- Poly(ADP-ribose) aka PAR
- PAR is a polymer that forms in many contexts, including DNA repair
- Catalyzed by PARP
- Digested by PARG

PAR

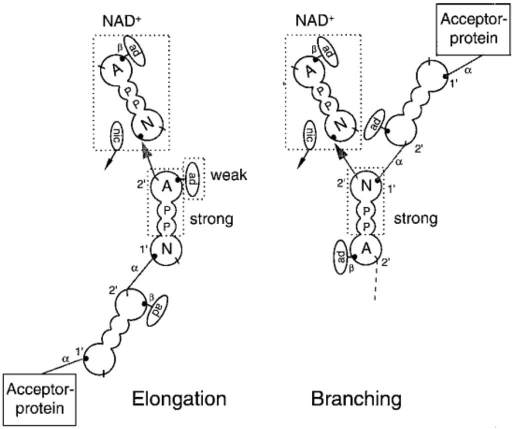
- Proposed as very general stress response to temperature, inflammation, cancer, neurodegeneration...
- Has a role in DNA repair, apoptosis, DNA regulation, RNA regulation, protein complex assembly...
- Potentially forms spontaneous vesicles (liquid demixing)



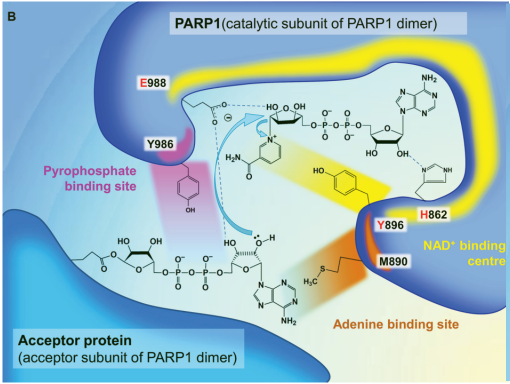
PARG

- PARG cuts PAR trees by severing NAD-NAD bonds
- PARG inhibitors used in cancer treatments
- At first glance PARG just cleans up after PAR is done
- But decreased activity of PARG *delays* DNA repair and makes cells more susceptible to DNA damage

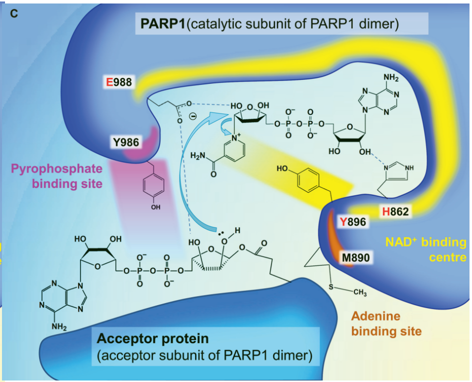
Elongation and Branching



Elongation and Branching



(a) Elongation



(b) Branching

Size Dependence on [NAD]

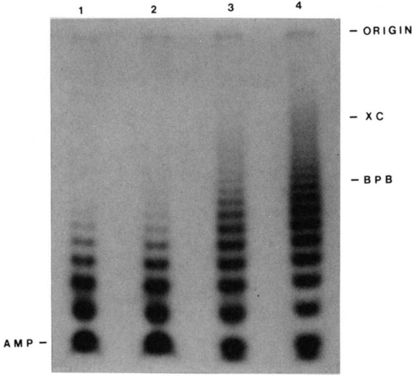


FIG. 2. **Size distribution of ADP-ribose polymers synthesized with 1.8 pmol of pure enzyme at 200, 400, 800, and 1600 nM [NAD] (*lanes 1, 2, 3,* and *4,* respectively).** The electrophoretic migration of xylene cyanol (*XC*) ((ADP-ribose)$_{20}$) and BPB ((ADP-ribose)$_8$) are indicated to the *right*, and AMP to the *left* of the autoradiograph.

Branching Ratio

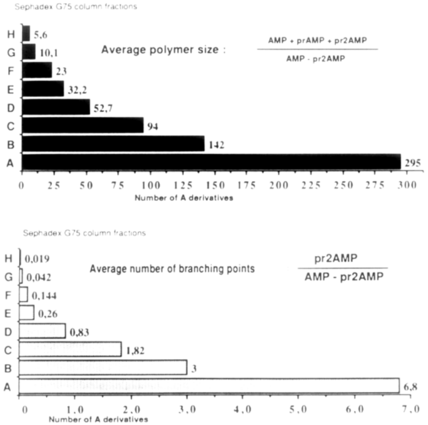


FIG. 5. Average polymer size and branching points of poly(ADP-ribose) fractions. A to H: fractions from G75 Sephadex column. The values were obtained by using the formulas shown.

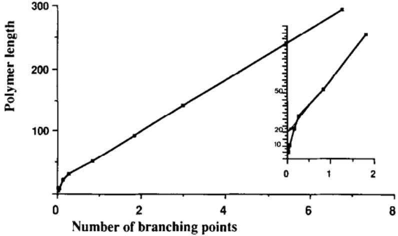


FIG. 6. Plot of polymer lengths versus number of branching points. The values correspond to those of Fig. 5.

The Kappa Model

Reactions

- Initiation: PARP binds to DNA to become active
- Should have 1 PARP per tree

- PARP can bind NAD in acceptor position or donor position
- doner NAD: leaf of a growing NAD tree
- acceptor NAD: free NAD+

- PARG cuts any two bound NADs
- All NAD chains separated from tree are digested at infinite rate

Elongation and Branching

- Donor side has pyrophosphate (P) and adenine (A) sites for PARP-NAD binding
- P binds independently, but A requires P already bound

- Elongation is more frequent.. and yet it requires more bonds
- $Pr(A\ P|\bar{A}\ P) >> k_{catalysis} \cdot Pr(\bar{A}|A)$
- Consistent with unimolecular binding rates

Implementation

- Model in Kappa
- Housed in Kappy (python)
- Parameter variation via string replacement
- Plotting using matplotlib

Simulation Results

[NAD] dependence

NAD concentration dependence without PARG.

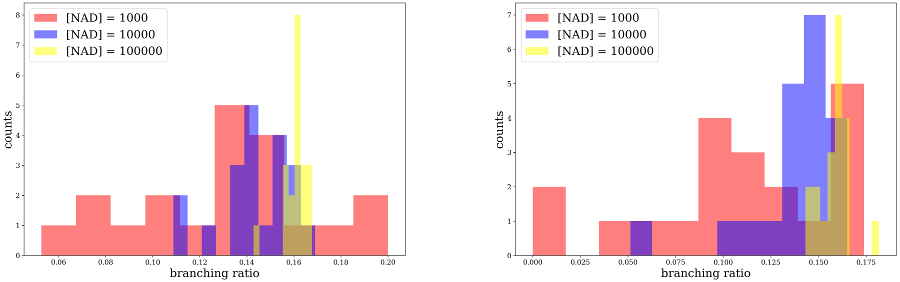
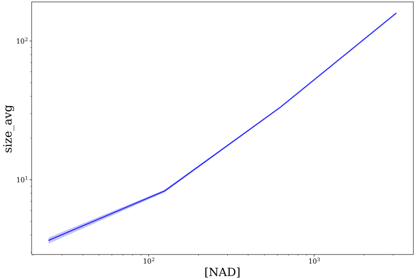


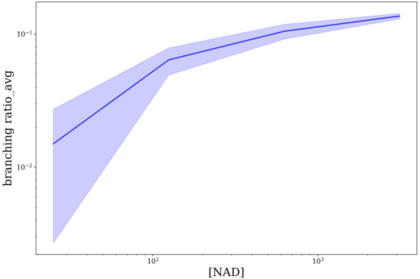
Figure: Branching ratios of all trees in absence of cutting mechanism

[NAD] dependence

NAD concentration dependence without PARG.



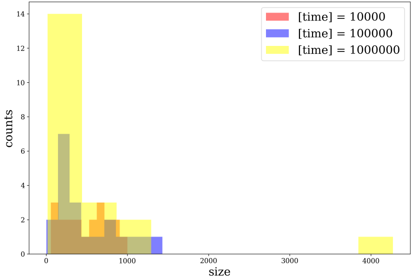
(a) Average tree size



(b) Average tree branching ratio

Tree size over time

Evolution of tree size with time.



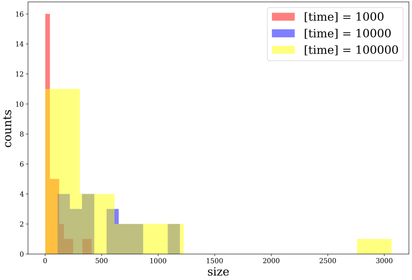
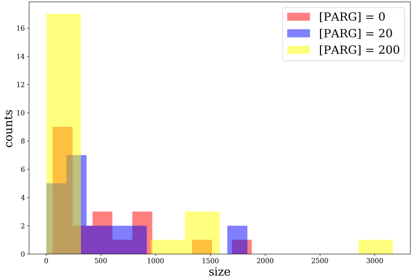
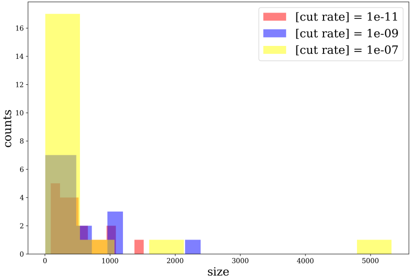


Figure: Size of all trees for different times ([NAD]=10^4)

PARG and Size



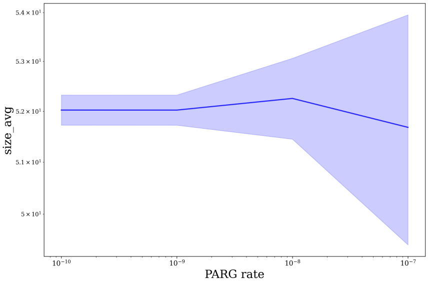
(a) Tree sizes for different [PARG]



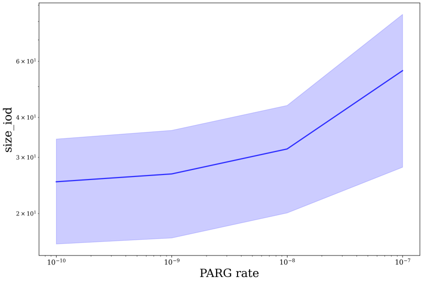
(b) Tree sizes for different cut rates

PARG and Size

PARG kinetic rates with [PARG] = 20.

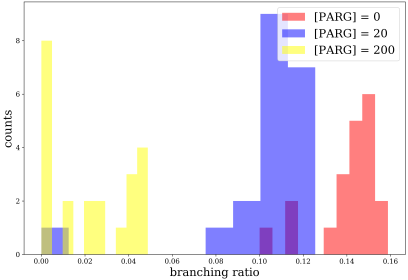


(a) Average tree size

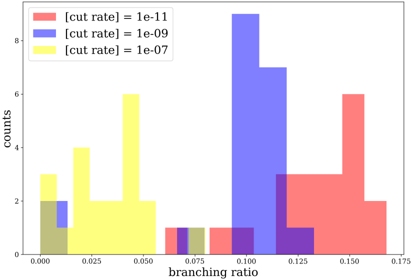


(b) Index of Dispersion $= \frac{\sigma^2}{\mu}$ of tree sizes

PARG and Branching



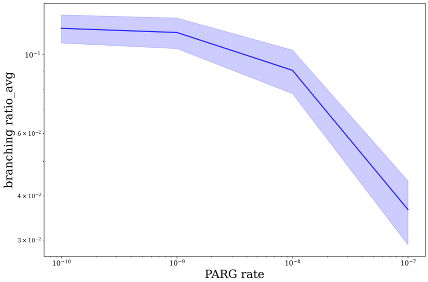
(a) Tree branching ratios for different [PARG]



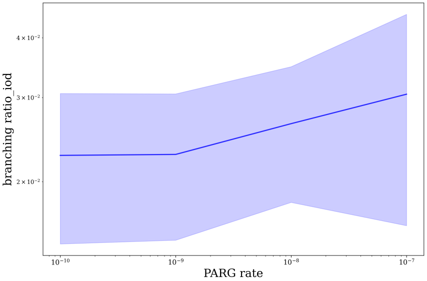
(b) Tree branching ratios for different cut rates

PARG and Branching

Various PARG kinetic rates for [PARG] = 20.



(a) Average tree branching ratio



(b) IOD $= \frac{\sigma^2}{\mu}$ of tree branching ratio

References

- Keith, G., J. Desgres, and G. De Murcia. "Use of two-dimensional thin-layer chromatography for the components study of poly (adenosine diphosphate ribose)." Analytical biochemistry 191.2 (1990): 309-313.
- Mendoza-Alvarez, Hilda, and Rafael Alvarez-Gonzalez. "Poly (ADP-ribose) polymerase is a catalytic dimer and the automodification reaction is intermolecular." Journal of Biological Chemistry 268.30 (1993): 22575-22580.
- Pascal, John M., and Tom Ellenberger. "The rise and fall of poly (ADP-ribose): an enzymatic perspective." DNA repair 32 (2015): 10-16.
- Altmeyer, Matthias, et al. "Liquid demixing of intrinsically disordered proteins is seeded by poly (ADP-ribose)." Nature communications 6 (2015): 8088.
- McGurk, Leeanne, et al. "Poly (ADP-ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization." Molecular cell 71.5 (2018): 703-717.

References

- McGurk, Leeanne, et al. "Poly (ADP-ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization." Molecular cell 71.5 (2018): 703-717.
- Alemasova, Elizaveta E., and Olga I. Lavrik. "Poly (ADP-ribosyl) ation by PARP1: reaction mechanism and regulatory proteins." Nucleic acids research 47.8 (2019): 3811-3827.
- Ruf, Armin, et al. "The mechanism of the elongation and branching reaction of poly (ADP-ribose) polymerase as derived from crystal structures and mutagenesis." Journal of molecular biology 278.1 (1998): 57-65.
- Luo, Xin, and W. Lee Kraus. "On PAR with PARP: cellular stress signaling through poly (ADP-ribose) and PARP-1." Genes development 26.5 (2012): 417-432.
- Houl, Jerry H., et al. "Selective small molecule PARG inhibitor causes replication fork stalling and cancer cell death." Nature Communications 10.1 (2019): 1-15.