THERMOCYCLING DRIVES EVOLUTION OF RNA GRANULES

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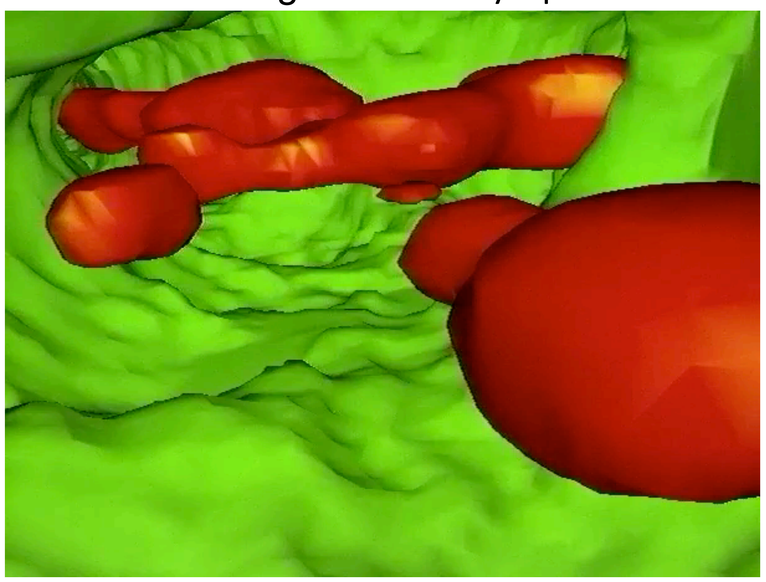
Abstract

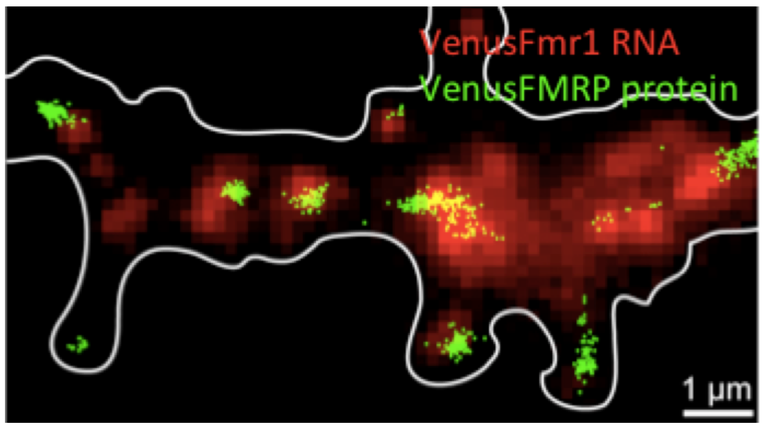
RNA granules represent a fundamental organizing principle of living cytoplasm. RNA granules are liquid droplets formed by phase separation of RNA and protein molecules. In modern cells mRNA molecules are localized and translated in RNA granules and their encoded protein molecules are associated with the same granules. We discovered that translation in individual RNA granules is cyclic, with periods of active translation interspersed with periods of translation inactivity. Active translation generates heat energy resulting in thermocycling in individual granules. Thermocycling regulates single stranded and double stranded RNA content, off rates for molecular binding and thermophoretic movement of protein and RNA molecules into and out of individual granule.

Primordial RNA granules may have formed prior to the appearance of living cells by phase separation of oligoribonucleotides and oligopeptides. If primordial granules formed near hydrothermal vents, temperature fluctuations (thermocycling) in the vent may have caused thermostatic switching between translation and replication, thermokinetic selection for slower off rates and thermophoretic selection for longer RNA molecules in the granules. Thus, thermocycling near hydrothermal vents may have driven evolution of selectivity, stability, complexity and information content in primordial RNA granules.

RNA granules in cytoplasm



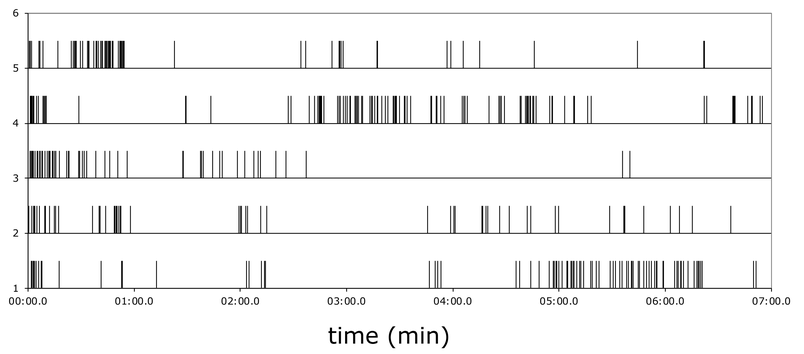
Translation output from RNA granules



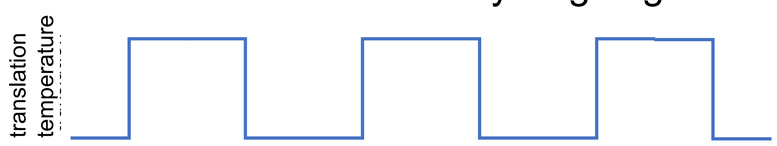
back-of-the-envelope calculations

- translation of one protein molecule uses ~ 2000 ATP/GTP molecules
- one burst of translation uses ~ 10^{7} ATP/GTP molecules/1-2min
- hydrolysis of one ATP/GTP molecule generates ~ 10^{-19} J of energy
- one burst of translation could generate ~ 10^{-12} J
- the volume of one RNA granule is ~ 10^{-15} l
- heating 10^{-15} l of water by 1°C requires ~ 10^{-12} J of energy
- one burst of translation could increase granule temperature by ~1° C

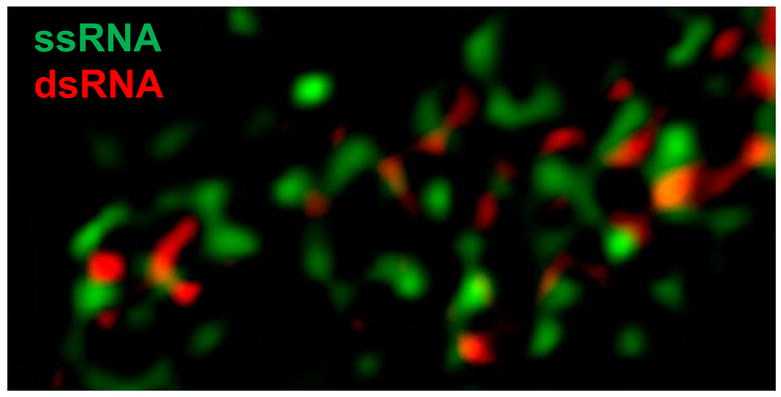
Translation events in granules



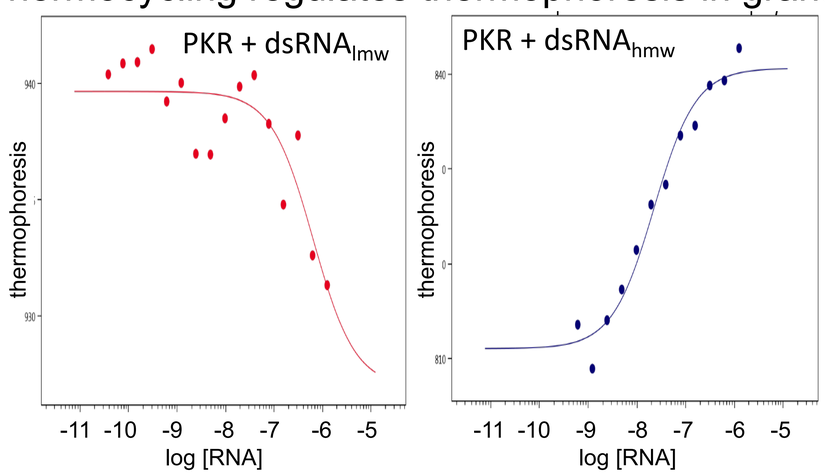
Translation drives thermocycling in granules



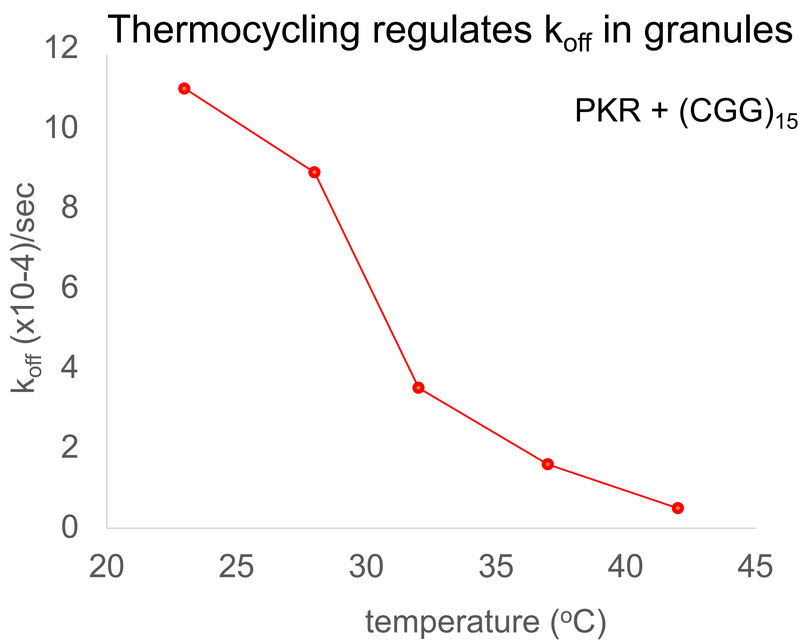
Thermocycling regulates ds/ss RNA in granules



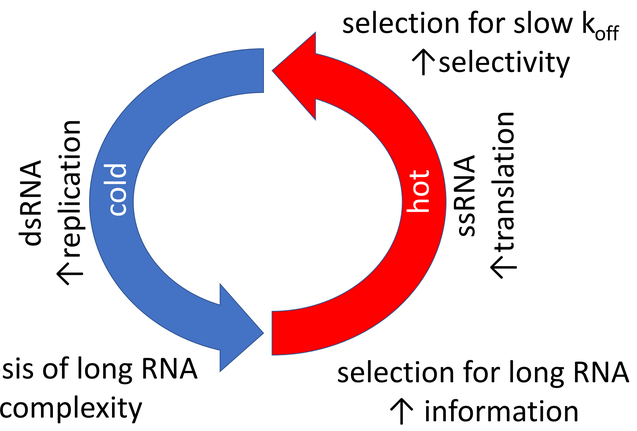
Thermocycling regulates thermophoresis in granules



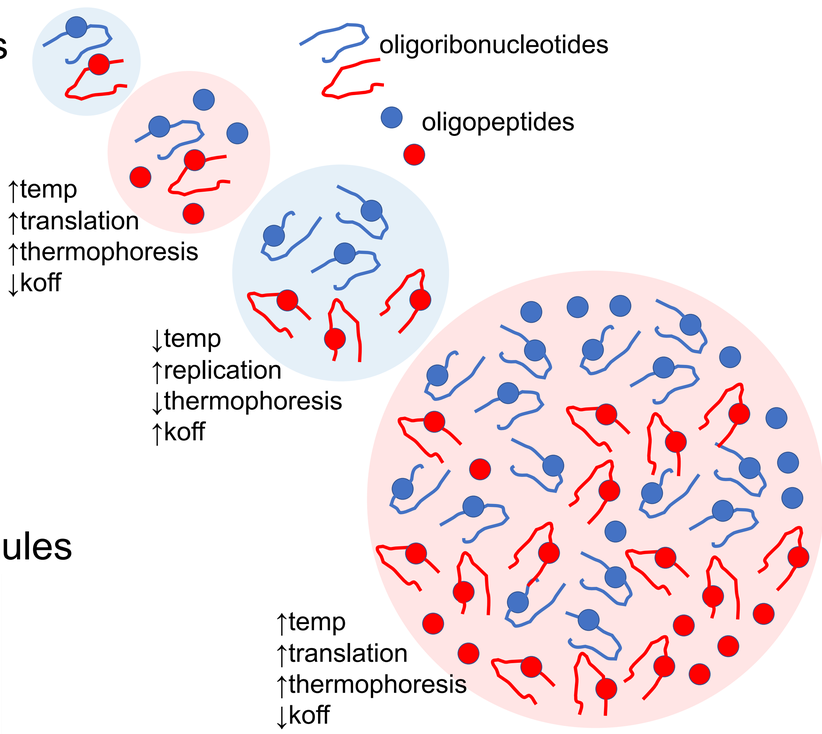
Thermocycling regulates k_{off} in granules



Thermocycling regulates structure and function of granules



Hypothesis: thermocycling drives evolution of primordial granules



Hypothesis testing: does thermocycling drive evolution of primordial granules in vitro

1. incubate random sequence oligoribonucleotides and oligopeptides to form primordial granules.
2. Incubate primordial granules with *in vitro* translation/RNA polymerase components (+/- inosine to increase mutation rate, + Venus RNA as a translation reporter, + ss/dsRNA fluorescent dyes).
3. incubate in qPCR machine to cause thermocycling in primordial granules.
4. analyze ss/ds RNA switching and RNA replication during thermocycling using ss/dsRNA fluorescent dyes.
5. analyze translation during thermocycling using Venus fluorescence.
6. analyze evolution of complexity, information, and stability of granules after multiple rounds of thermocycling using RNAseq and FCS/FRAP.