In Vivo Dimerization of Group II Intron Ribonucleoprotein Complexes Precedes Homing-Site Invasion During Retrohoming

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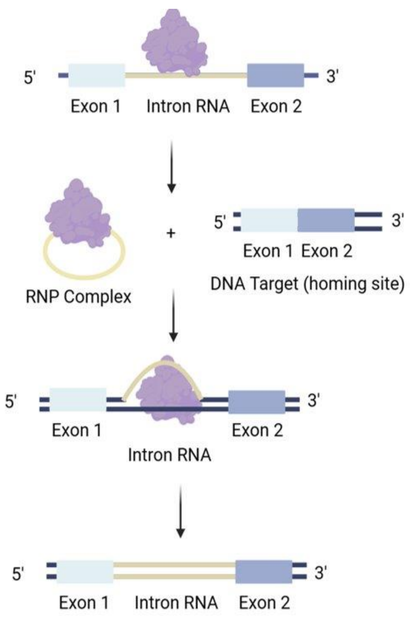
undergraduate science showcase

Introduction



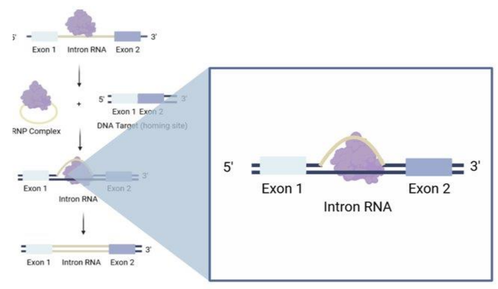
Group II introns are **self-splicing** RNA ezymes that also function as mobile retroelements in bacteria and organelles of eukaryotes.

Their mobility depends on a **ribonucleoprotein (RNP)** complex composed of the spliced intron RNA and a multifuntional intron-encoded protein (IEP).

The IEP promotes RNA splicing and contains endonuclease and reverse transcriptase domains required for insertion into a specific DNA homing site (HS).

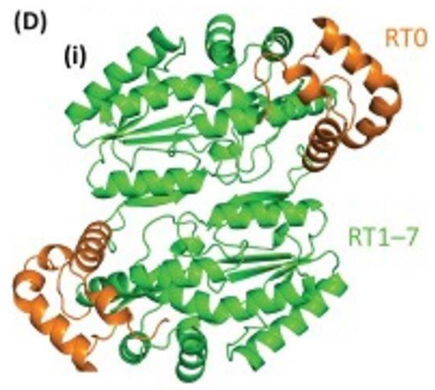
The **Ll.LtrB** intron and **LtrA** IEP from *Lactococcus lactis* is a well-established model system for studying group II intron structure, assembly, and mobility.

Current Group II Intron Retromobility Model



The current retromobility model assumes a **single** RNP acts at the DNA homing site

BUT...



Crystallography showed the **dimerization** of two IEPs consistent with unpublished biochemical data from the Cousineau lab

Hypothesis

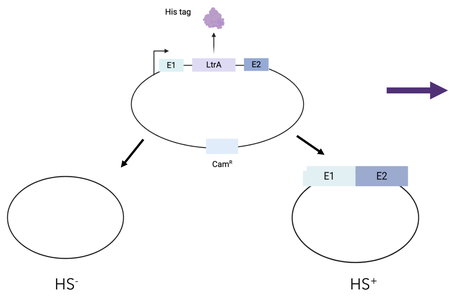
Group II intron RNPs form **dimers** before DNA recognition and invasion at their homing sites.

Aim

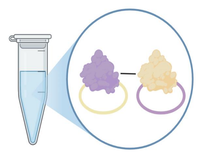
To study RNP dimers *in vivo* in the presence or absence of the homing site.

Methods

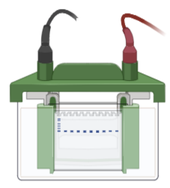
1. Co-express with HS^+/HS^-



2. DSS crosslink



3. Western blot



Results

Absence of Homing Site

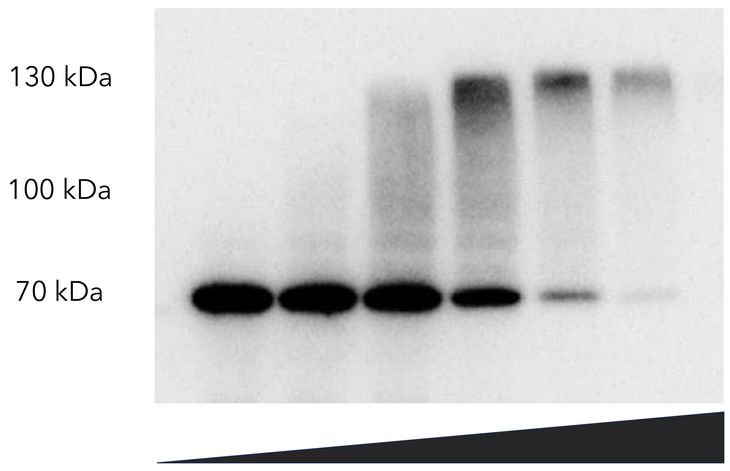


Figure 1. LtrA forms dimers *in vivo* in a DSS dose-dependent manner. *Lactococcus lactis* cells containing a plasmid expressing Ll.LtrB–WT–LtrA–His ($M^+/E^-/RT^-$) in the absence of the homing site (HS^-) were grown to exponential phase and treated with increasing concentrations of DSS (0, 0.01625, 0.03125, 0.06250, 0.12500, and 0.25000 mM) prior to lysis. Total cell extracts were analyzed by Western blot using an anti-His antibody, revealing monomeric (~70 kDa) and dimeric (~130 kDa) LtrA species.

Presence of Homing Site

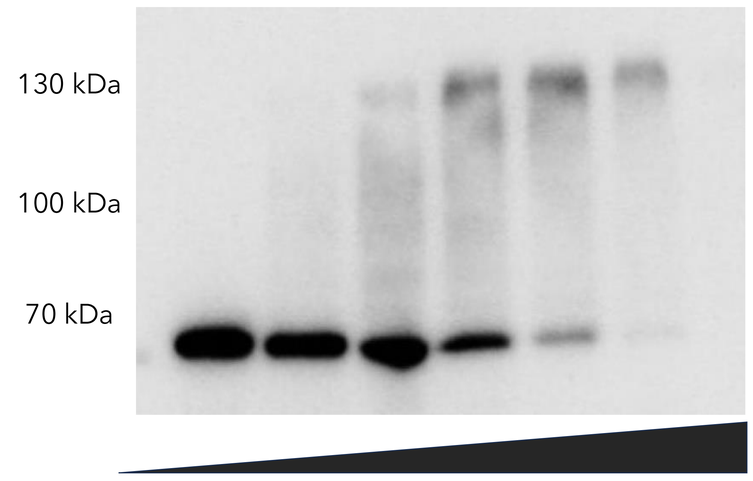
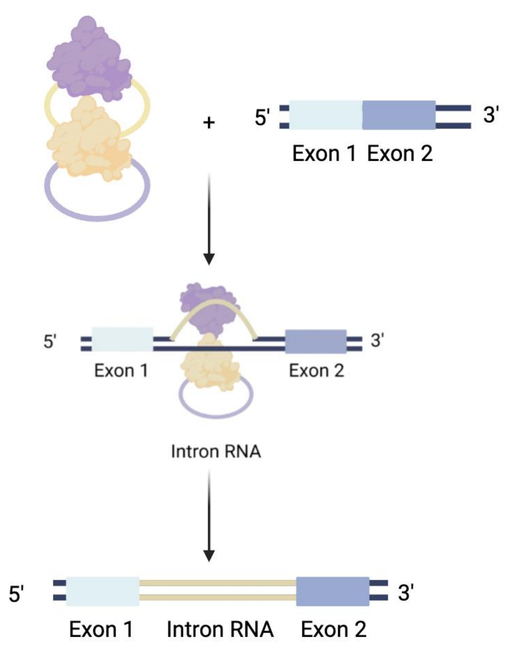


Figure 2. LtrA forms dimers *in vivo* in a DSS dose-dependent manner. *Lactococcus lactis* cells containing a plasmid expressing Ll.LtrB–WT–LtrA–His ($M^+/E^-/RT^-$) in the presence of the homing site (HS^+) were grown to exponential phase and treated with increasing concentrations of DSS (0, 0.01625, 0.03125, 0.06250, 0.12500, and 0.25000 mM) prior to lysis. Total cell extracts were analyzed by Western blot using an anti-His antibody, revealing monomeric (~70 kDa) and dimeric (~130 kDa) LtrA species.

Conclusions

- Group II intron RNPs form **dimers** *in vivo*.
- Dimerization occurs **independently** of homing-site recognition, suggesting it **precedes** DNA target invasion.
- Studying *in vivo* assembly of group II intron RNPs provides insight into the **evolution** of RNA–protein complexes and informs the development of novel **biotechnological** tools.



References

Acknowledgements

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