

Background

Ancestral Sequence Reconstruction uses the sequences of extant proteins to predict the sequences of ancestral ones.

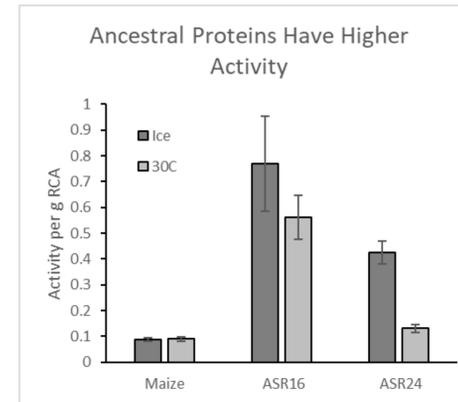
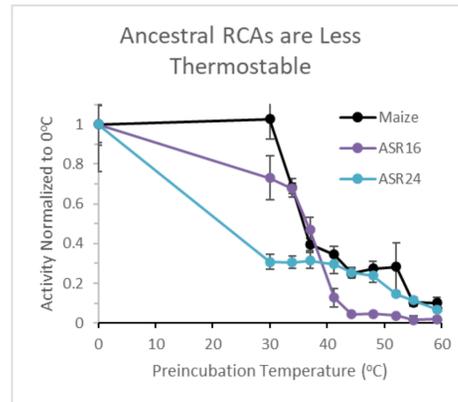
Ancestral proteins are often **more stable**, more promiscuous, and/or more active¹

Rubisco Activase (RCA) is a chaperone for Rubisco, but **is not thermostable**. It inhibits photosynthesis at high temperature².

We reconstructed RCA and compared its thermostability to the protein from *Zea mays* (maize).

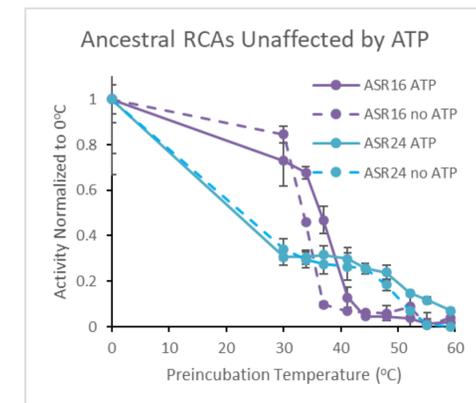
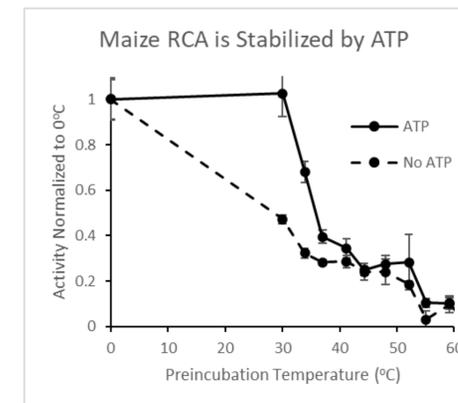
Thermostability

Ancestral sequences have higher activity but lower thermostability



Purified protein was incubated at indicated temperatures for 1hr
 Activity was measured by a spectrophotometric ATPase assay⁵

Ancestral proteins are not stabilized by ATP



Extant RCA proteins are known to have improved thermostability when incubated with ATP⁶
 This may be an evolutionary innovation rather than an ancestral trait

Reconstruction

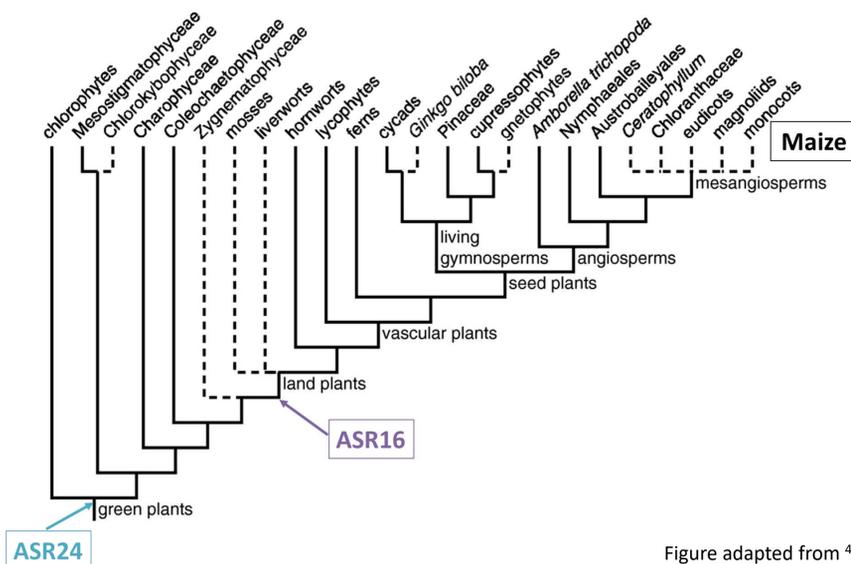


Figure adapted from ⁴

1	Zea mays	MAAEVDETKQTDDEDRWGL--AYDISDDQDDITRGGKLVNLFQAPM--GDGTHVAVLSSYDYISQGGKSYNFDNMM	78
	ASR16	---MEVDETKQTKDRWGL--GYDSDDDQDDITRGGKLVNLFQAPM--GSGTQNAVMSYBYISQGGKSYNFDNMM	
	ASR24	---MAEVDETKQTKDRWGL--AYDISDDQDDITRGGKLVNLFQAPM--GDGTHVAVLSSYDYISQGGKSYNFDNMM	
79	Zea mays	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESNGAGEPAKLI	156
	ASR16	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESNGAGEPAKLI	
	ASR24	DGFYIAKGFMDKLVVHLSKNFMFLPNIKVPLILGIWGGKGGKSFQCELVFAKMGITPIMMSAGELESNGAGEPAKLI	
157	Zea mays	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEPRVPIIIVT	234
	ASR16	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEPRVPIIIVT	
	ASR24	RQRYREASDLIK-KGKMSCLFINDLDAGAGRMGGTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKEPRVPIIIVT	
235	Zea mays	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	312
	ASR16	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	
	ASR24	GNDFSTLYAPLIRDGRMEKFWAPTREDRIGVCKGIFRTDGVDEEHVQLVDTFPGQSIDFFGALRARVYDDEVRWV	
313	Zea mays	SETGVENIARKLVNSKEGPTTFEPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	388
	ASR16	SETGVENIARKLVNSKEGPTTFEPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	
	ASR24	SETGVENIARKLVNSKEGPTTFEPKPTIEKLLYGHMLVAEQENVKRVQLADKYLNAAALGEANEDAMKTGSFFK	

Conclusions

- Reconstructed ancestral proteins were highly active but not thermostable compared to maize
 This breaks the trend that ancestral sequences are usually more stable
- Maize RCA is thermostable and may not be an informative comparison
 Future work should compare thermostability to RCA from other species such as spinach or Arabidopsis
- Stabilization of RCA by ATP is not an ancestral trait but begins to appear in land plants.

References

- Spence MA, Kaczmarek JA, Saunders JW, Jackson CJ. Ancestral sequence reconstruction for protein engineers. *Curr Opin Struct Biol.* 2021;69:131-141. doi:10.1016/j.sbi.2021.04.001
- Crafts-Brandner SJ, Salvucci ME. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂. *Proc Natl Acad Sci U S A.* 2000;97(24):13430-13435. doi:10.1073/pnas.230451497
- Musil M, Stourac J, Bendl J, et al. FireProt: web server for automated design of thermostable proteins. *Nucleic Acids Res.* 2017;45(W1):W393-W399. doi:10.1093/nar/gkx285
- Davis CC, Xi Z, Mathews S. Plastid phylogenomics and green plant phylogeny: Almost full circle but not quite there. *BMC Biol.* 2014;12:2-5. doi:10.1186/1741-7007-12-11
- Barta C, Carmo-Silva AE, Salvucci ME. Rubisco Activase Activity Assays. In: *Photosynthesis Research Protocols.* Vol 684. ; 2011:375-382. doi:10.1007/978-1-60761-925-3
- Keown JR, Pearce FG. Characterization of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase activase isoforms reveals hexameric assemblies with increased thermal stability. *Biochem J.* 2014;464(3):413-423. doi:10.1042/BJ20140676

- The common ancestors of land plants and green plants were reconstructed using the FireProt webserver³
- Alignment shows the sequence is significantly conserved
- Proteins were expressed in *Escherichia coli* and purified by Ni-NTA affinity chromatography