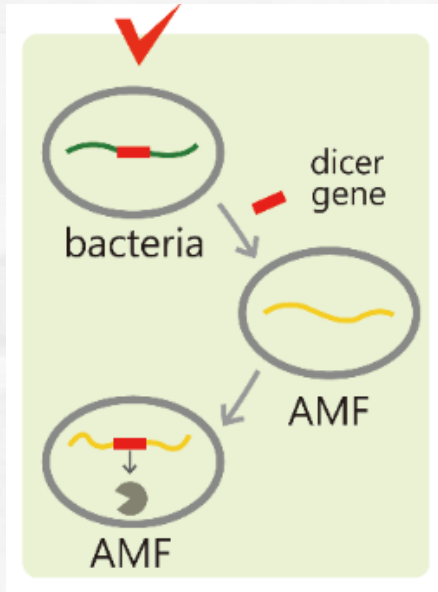


Conserved proteins in RNA interference system of AMF provide new insight into the evolutionary history of *Glomeromycota*

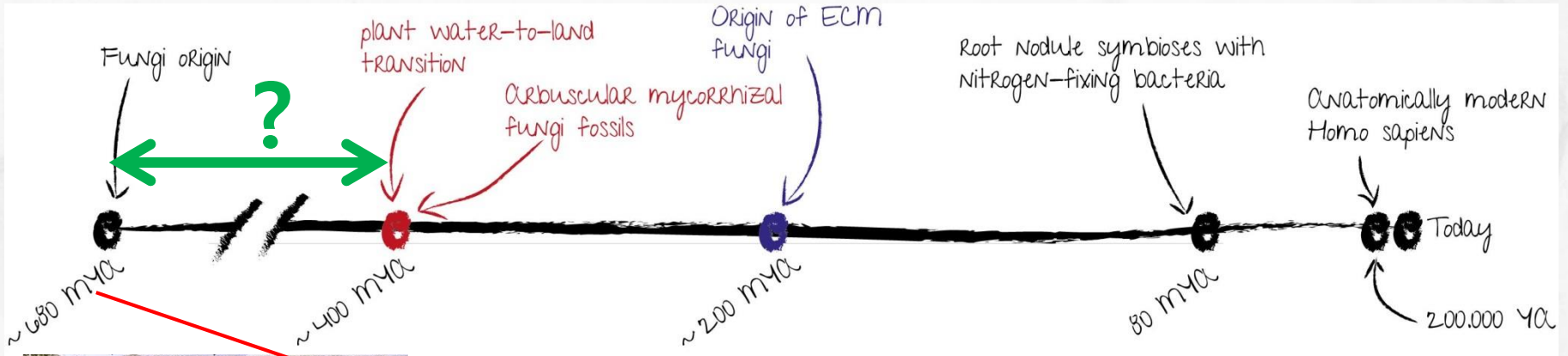


Mycorrhizes 2017

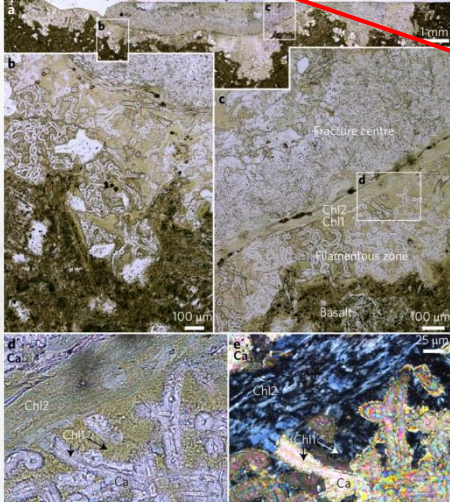
SOON-JAE LEE

Labo Hijri

Evolution of Plant-microbes symbiosis



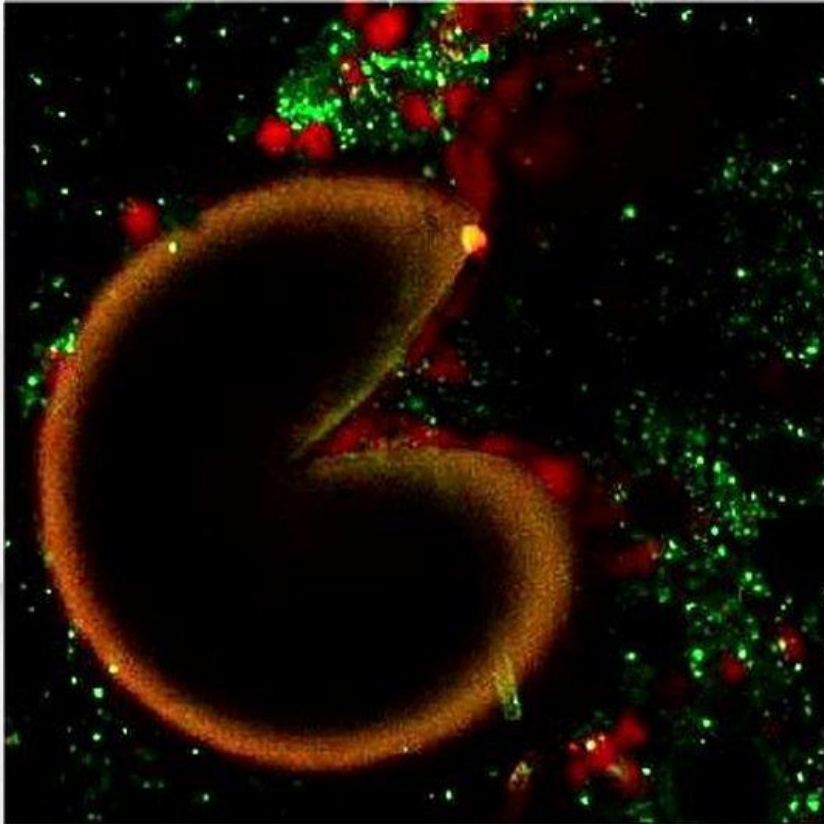
Recent Advances in Proteomics Research
Published by InTech (2015)



Bengtson et al (2017)

~2.4 bya (?)

The glimpse of co-evolution between fungal and bacterial ancestor by Horizontal Gene Transfer (HGT)



Mollicutes-related endobacteria in some AMF species

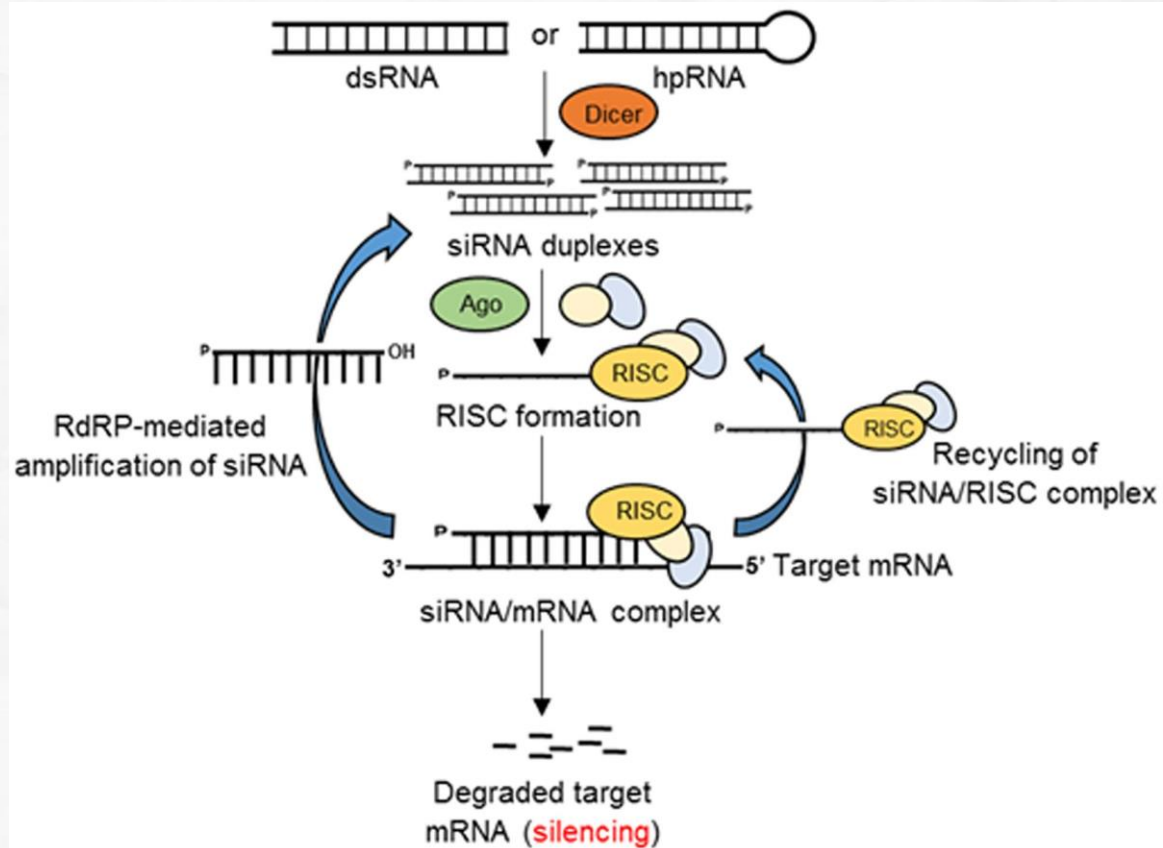
3 - 5 % of genes of Prokaryotic endosymbionts were acquired by HGT from Fungi (homologs from *Glomeromycota* and *Mucoromycotina*)

RNA interference and regulation of host gene expression during symbiosis

Host-symbiont crosstalk is mediated by **sRNA** as well as chemical signal.

Same in AMF-plant mycorrhization.
(Branscheid et al., 2011)

AMF-bacteria symbiosis?





Main objectives

To search the conservation of RNAi system in AMF

To search the trace of HGT in AMF genome

Work flow

Phase I

- In silico analysis to find RNAi system inside AMF
- EST and Transcriptome

Phase II

- Finding and expression confirming of HGT
- By Sanger sequencing and qPCR

Phase III

- In silico analysis to confirm the conservation of HGT

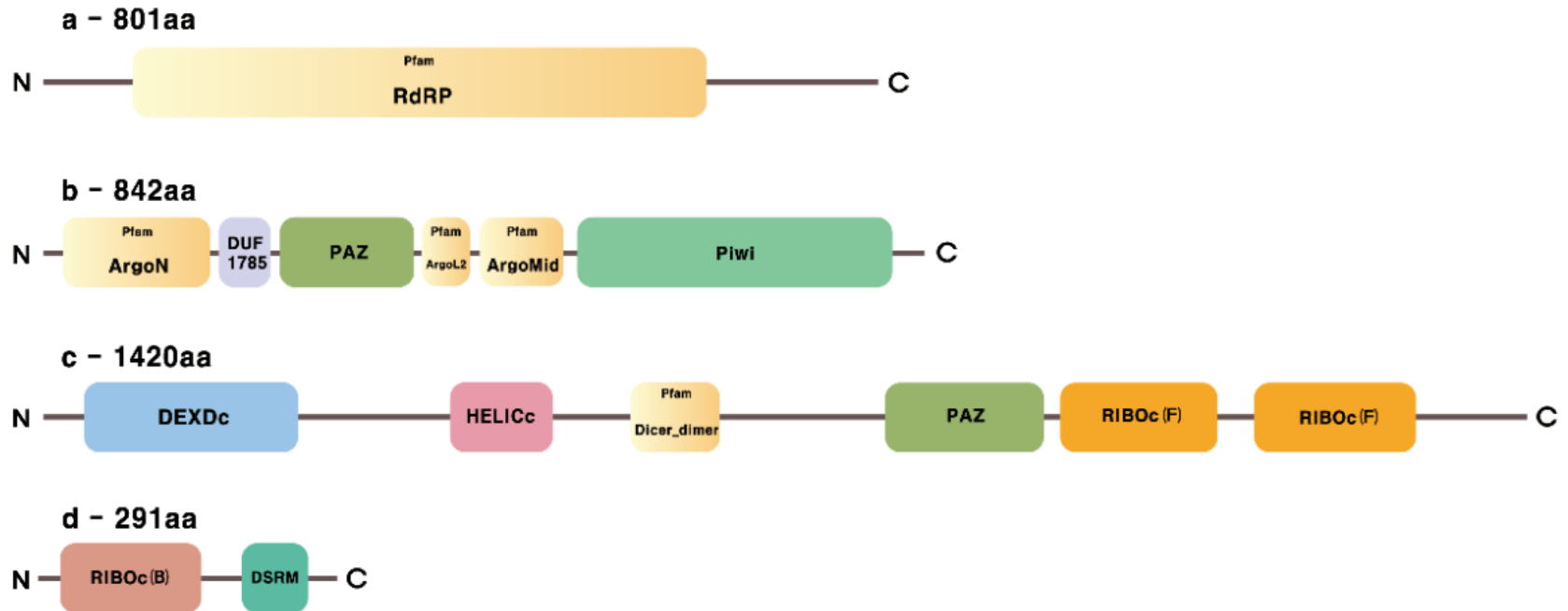
Results

AMF had all of 3 core components of RNAi system

Name of core component of RNAi system	The number of homologs in <i>R. irregulare</i>
RNA dependent RNA polymerase (RDRP)	4
Argonaute/Piwi Protein (ARGONAUTE/PIWI)	26
Ribonuclease III (DICER)	1 Fungal-origin ribonuclease III 2 Bacterial-origin ribonuclease III

Results

Protein domain architecture analysis





Results

All of three core fungal component protein homologs of AMF showed the highest homology with *Mucor circinelloides* (*Mucoromycotina*) in phylogenetic analysis

Results

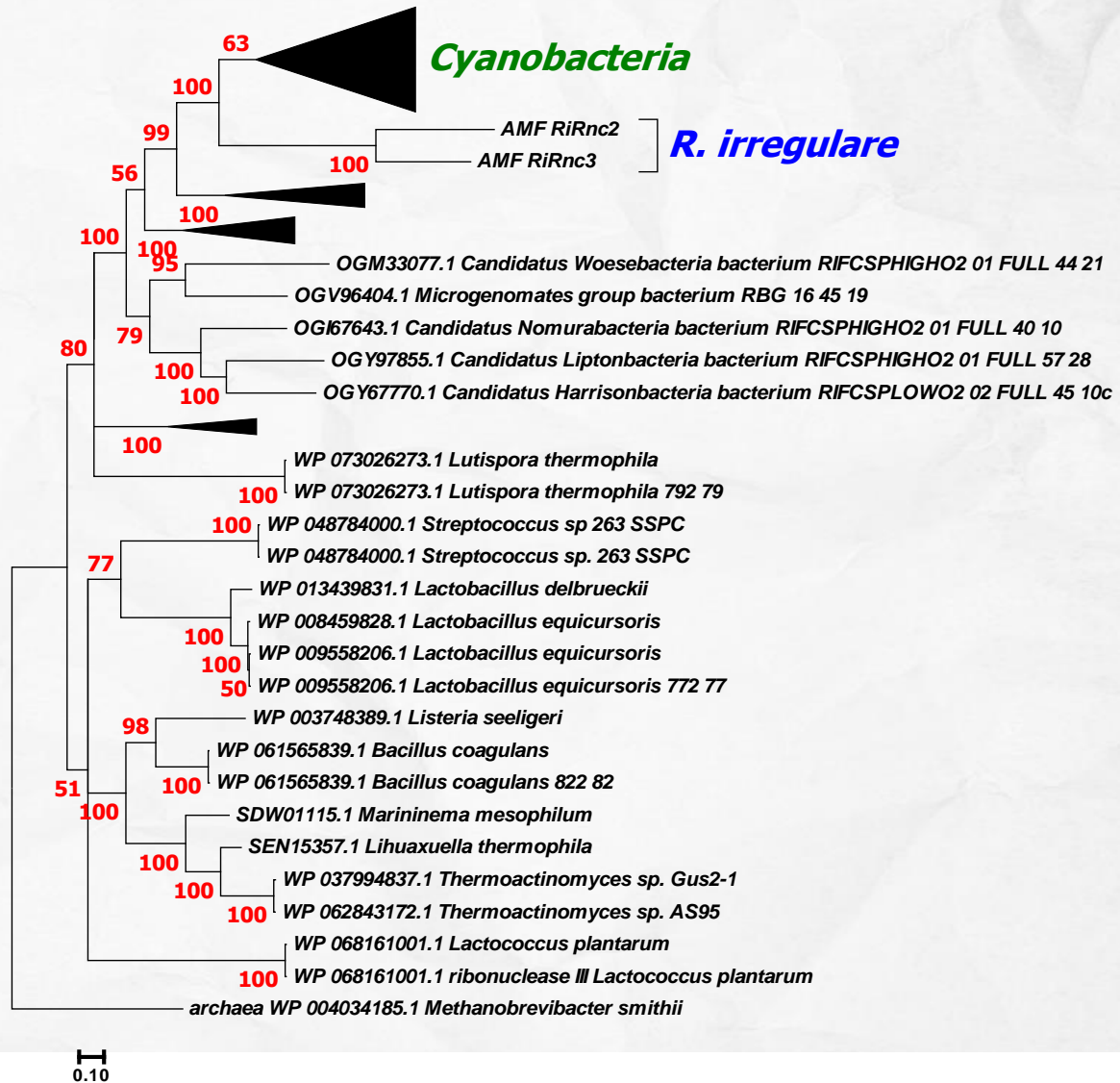
Naming of ribonuclease III

Fungal origin

- RIDCL1

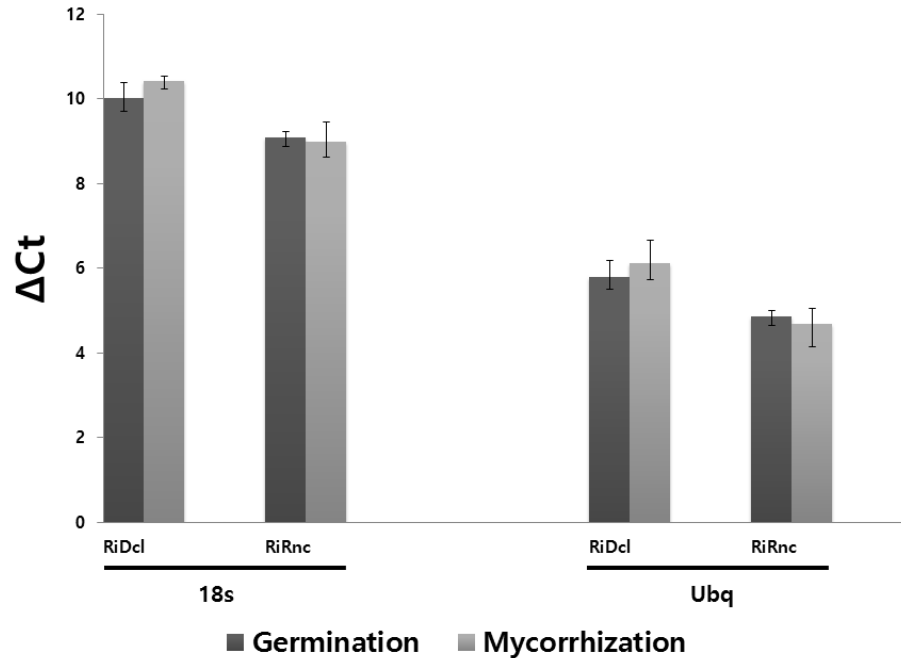
Bacterial origin

- RIRNC2
- RIRNC3



Results

1. cDNA based Sanger sequencing -> mRNA expression of *rirnc 2*
2. Expression of *ridcl 1* and *rirnc 2* (comparative Taqman assay)



under germination:
~1.9 fold
under mycorrhization:
~2.7 fold

$P < 0.05$

Results

High level of Catalytic domain (RIBOc) conservation among AMF

```

      10      20      30      40      50      60
S_pombe_PAC1      -----K L K K H A L M H I S R A Y E I Y P N Q S N P N E L L D I H N E R L E F L G D S F F N L F T T
S_cerevisiae_RNT1 -----T I K D K V Y L -----S G S --E M I N A H N E R L E F L G D S I L N S V M T
Rhizophagus_clarus -----K L R L E A L T H K S Y H Y E - N P S T G P -----H N E R L E F L G D S I V S F V V A
Rhizophagus_diaphanus -----K L R L E A L T H K S Y H Y E - N P S T G P -----H N E R L E F L G D S I V S F V V A
Rhizophagus_irregularis -----K L R L E A L T H K S Y H Y E - N P S T G P -----H N E R L E F L G D S I V S F V V A
Claroideoglomerum_etunicatum -----M E A L T H K S Y H Y E - N P S T G P -----H N E R L E F L G D S I V S F V V A
Scutelospora_calospora K K K T A R Q R N F N N D N L R L E A L T H K S F H Y E - N P K P G P -----H N E R L E F L G D S I V S F V V A
Microcoleus_sp_PCC7113 -----N L L R Q A L T H R S Y V N E - H P D A G E -----H N E R L E F L G D A V L G F L I G

      70      80      90      100     110     120
S_pombe_PAC1      R I I F S K F P Q M D E E S L S K L R A K F V G N E S A D K F A R L Y G F D K T L V L S Y S A E K Q Q L ---R K S Q K
S_cerevisiae_RNT1 L I I Y N K F P D Y S E G Q L S T L R M N L V S N E Q I K O W S I M Y N F H E K L K T N F D L K D E N S N F Q N G K L K
Rhizophagus_clarus -----N Y L H G R F P N F K E G Q L T L L R A N L V C K K K L A Q F A L Q L G L D K D I R L G V G A L R D G G ---R G S E -
Rhizophagus_diaphanus -----N Y L H G R F P N F K E G Q L T L L R A N L V C K K K L A Q F A L Q L G L D K D I R L G V G A L R D G G ---R G S E K
Rhizophagus_irregularis -----N Y L H G R F P N F K E G Q L T L L R A N L V C K K K L A Q F A L Q L G L D K D I R L G V G A L R D G G ---R G S E K
Claroideoglomerum_etunicatum -----N Y L F N R F P N F K E G Q L T L L R A N L V C K K K L A Q F A L Q L G -----
Scutelospora_calospora -----N Y L F G R F P N F K E G Q L T L L R A N L V C K K K L A Q F A L Q L G L D E D I R L G V G -----
Microcoleus_sp_PCC7113 -----E L L Y K R Y P E M S E A Q L T R L R S N L V D E K Q L A K F A T Q L G I G E L I R L G K G A I K E G G ---R E N P S

      130     140     150     160
S_pombe_PAC1      V I A D T F E A Y L G A L I L D G - Q ---E E T A F Q W S R L L Q P K I A N I T V
S_cerevisiae_RNT1 L Y A D V F E A Y I G G L M E D D P R N N L P K I R K W L R K L A K P V I E E A T R
Rhizophagus_clarus -----V L E D A F E A Y I G A V F L D A - G S S M S E V Q K F M E P L L A P A V D E L T H
Rhizophagus_diaphanus -----V L E D A F E A Y I G A V F L D A - G S S M S E V Q K F M E P L L A P A V D E L T H
Rhizophagus_irregularis -----V L E D A F E A Y I G A V F L D A - G S S M S E V Q K F M E P L L A P A V D E L T H
Claroideoglomerum_etunicatum -----
Scutelospora_calospora -----
Microcoleus_sp_PCC7113 -----L L S D T F E A Y I A A Y F I E S - G ---I E A V Q Q F V Q P L F T K V A D S I V F
```

residue 17 to 96, 80 a.a

Rhizoglomerum

- 80/80

Claroideoglomerum

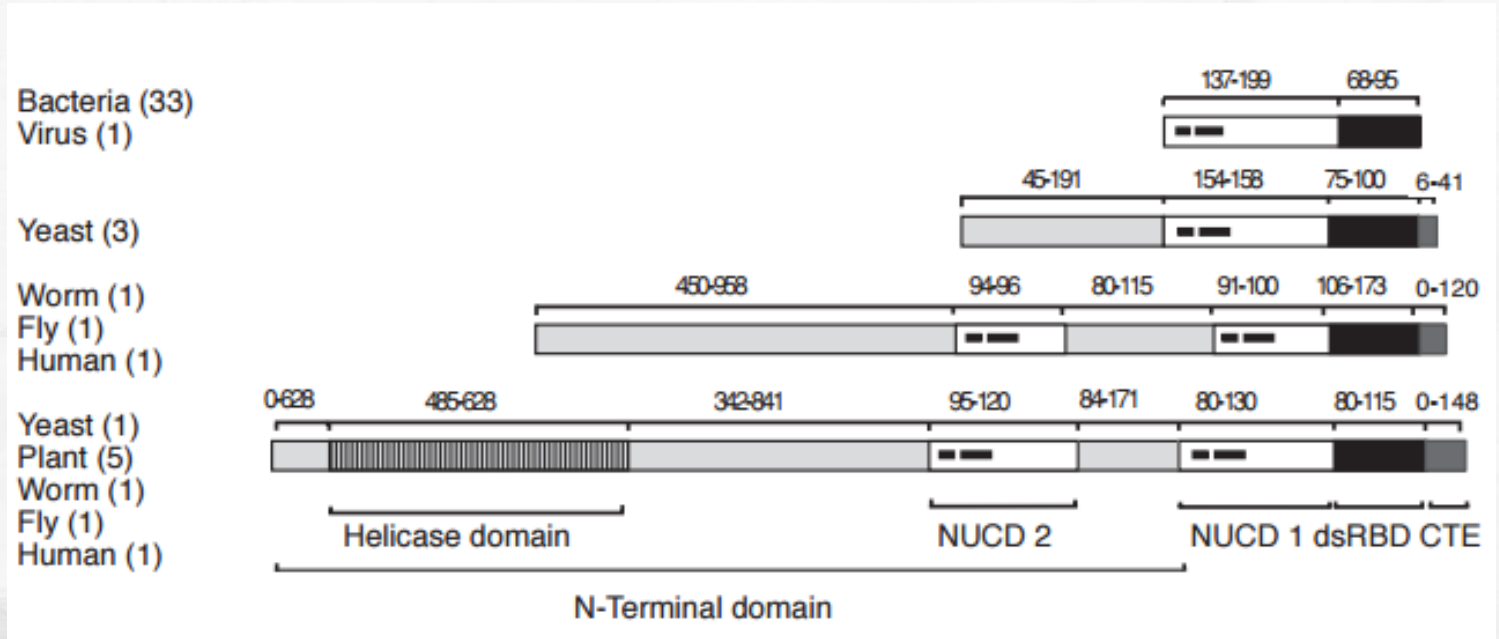
- 78/80

Scutelospora

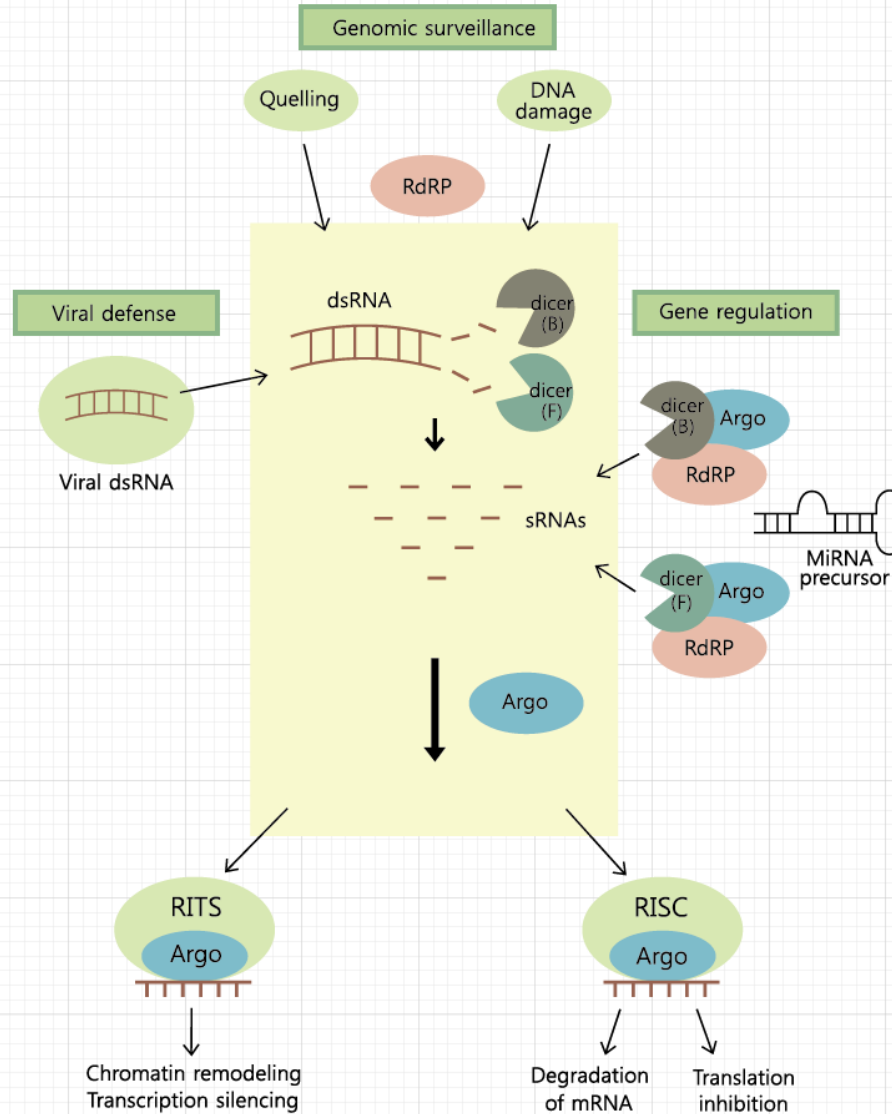
- 76/80

Glu-Phe-Leu-Gly-Asp
(from 48 to 52 a.a.)

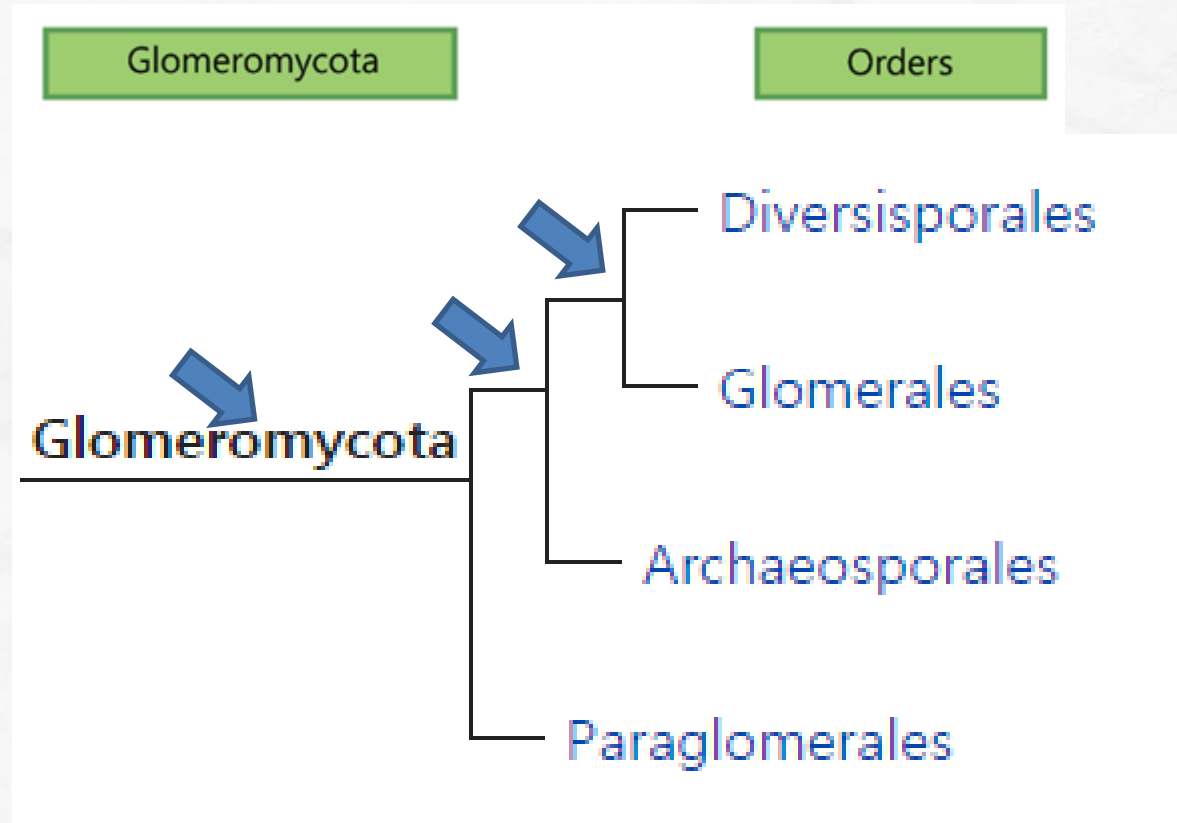
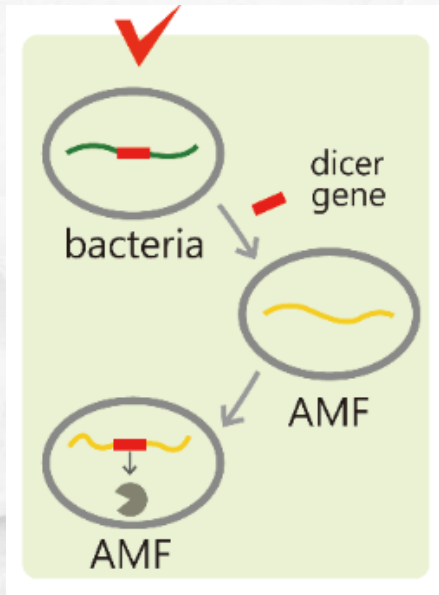
Conclusion



Conducion



Conduction



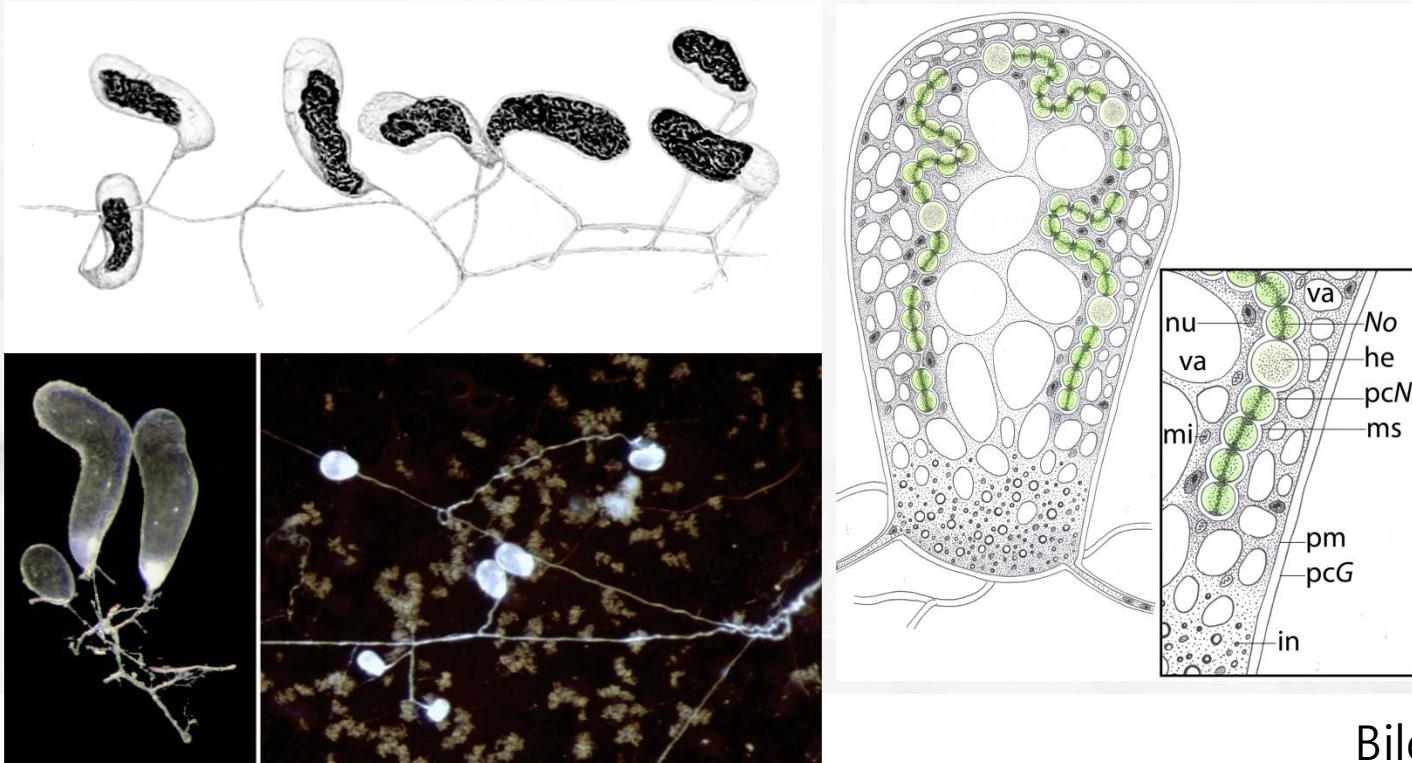
Conduction

1. *R. irregulare* has RNAi system core components conserved and expressed in mRNA level
2. *R. irregulare* has unique prokaryotic Rnase III coding gene (Rirnc 2 and Rirnc 3) horizontally transferred from cyanobacteria
3. The prokaryotic Rnase III resulted from HGT were conserved among AMF species

Condusion

Another evidence, living fossil of symbiosis between AMF and cyanobacteria.

Geosiphon pyriforme and *Nostoc punctiforme*

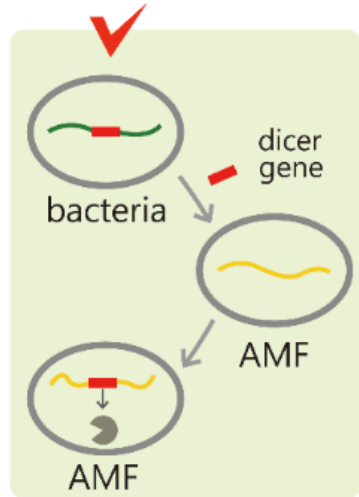
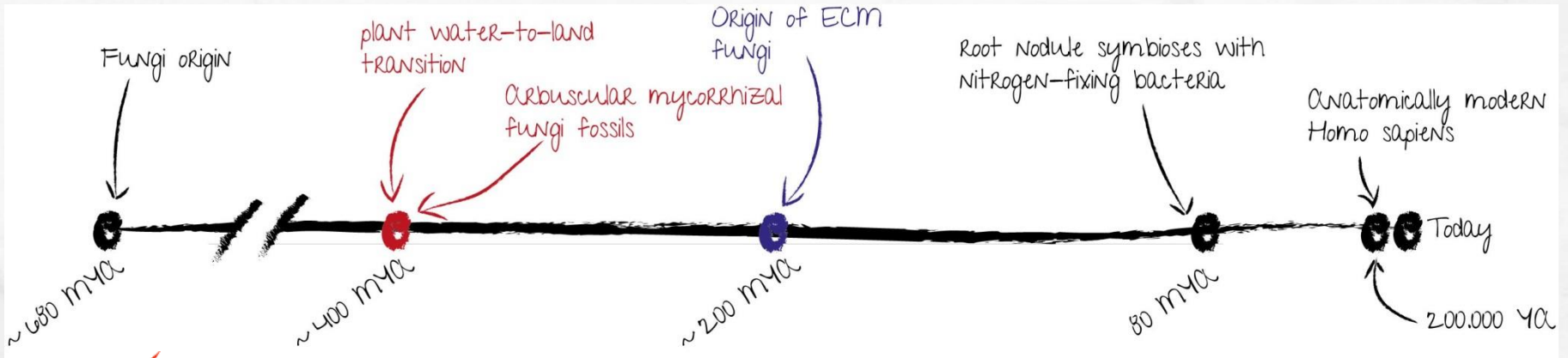




Future perspective

1. Development of Glomerales and Diversisporales specific primer.
2. Advanced research for the evolution of prokaryotic rnae III inside *glomeromycota*.
3. RNAi based approach for gene study of AMF.
4. RNAi based mycovirus defense system study of AMF.

Thank you for your attention



Questions?