ECFG15
ROME • ITALY 2020

université
PARIS-SACLAY

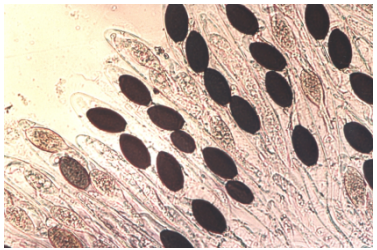
Epigenetics, RIP and sexual development in filamentous ascomycetes

Fabienne Malagnac

The fungal epigenetics lab project

- To understand the relationships between **epigenetic modifications** that shape **the chromatin structure** and :
  - **genome stability**
  - **gene regulation** during developmental processes (**sexual reproduction**)

✓ **Using filamentous fungi as model organisms**

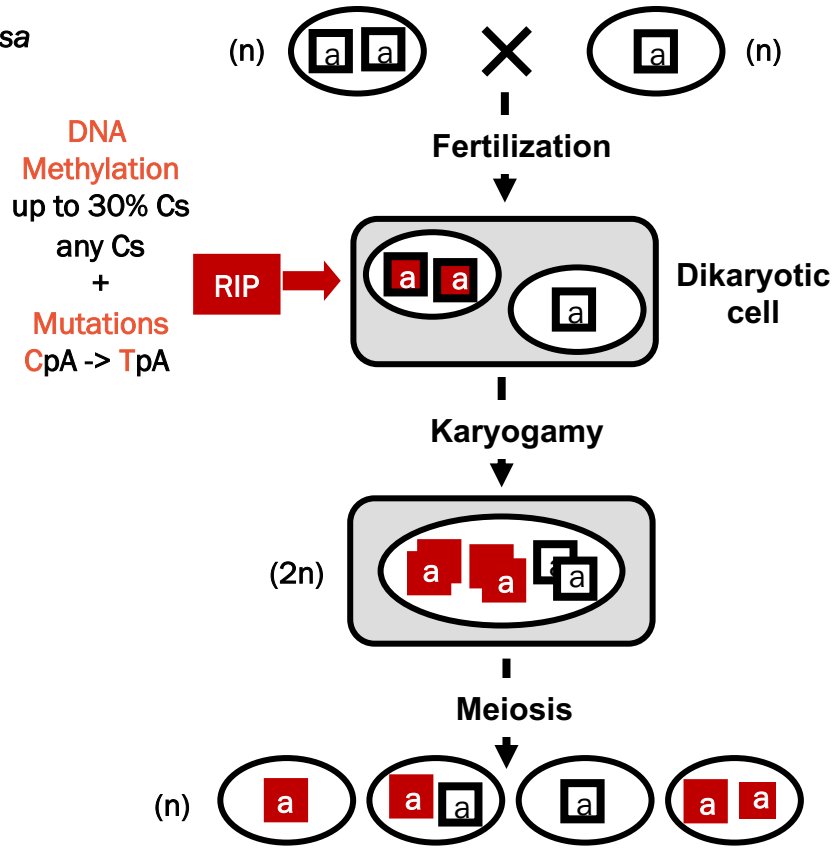


Podospora anserina

Ascobolus immersus

Repeat Induced Point Mutation (RIP)

Neurospora crassa



TARGETS

Repeats $N \geq 2$

=> TEs (~9% of *Neurospora* genome)

RIPed alleles :

methylated (silenced)

+

mutated (pseudo-genes)

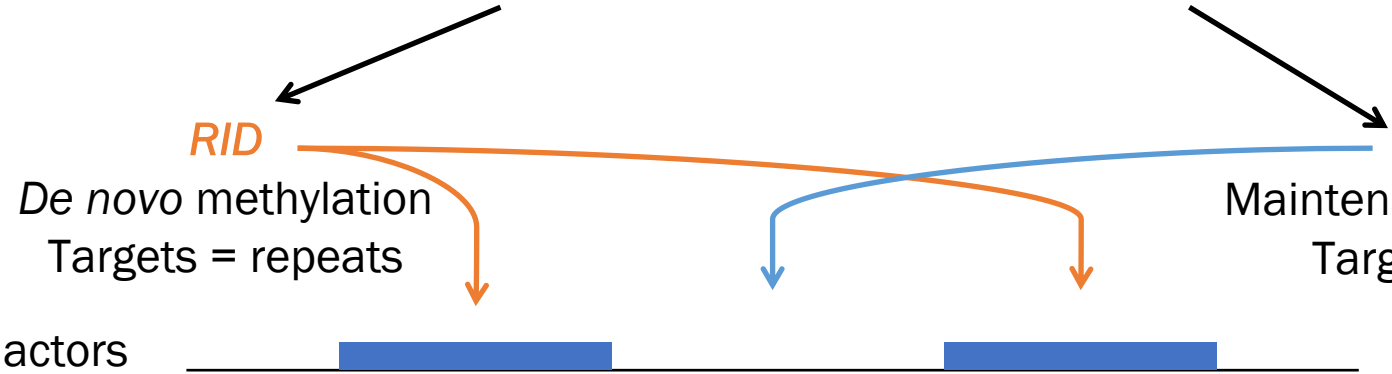
CONSEQUENCES

epigenetic AND genetic

Selker *et al.* (1987)

RIP in *N. crassa*

Two RIP effectors : DNA-methyltransferases



ΔRID mutant

NO *de novo* DNA methylation

NO RIP on repeats

ΔDim2 mutant

NO DNA methylation

NO RIP on the spacer

Freitag *et al.* (2002) ; Eugene Gladyshev & Nancy Kleckner, (2017)

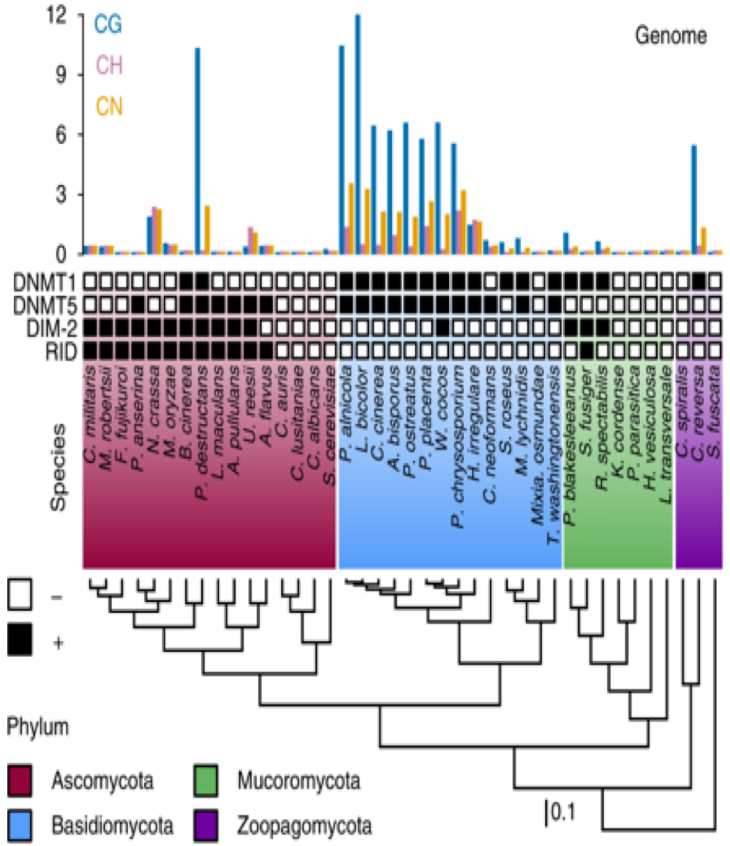
Are RIP / RID conserved ?

- **Traces of RIP are found in most Pezizomycotina genomes (*in silico*)**

➢ **RIP is important for fungal genome integrity and evolution**

- **RIP is conserved but :**
  - in most cases no 5mC
  - light mutagenic effect
- **RIP-like systems are polymorph**
- **RID protein is conserved in Pezizomycotina**

→ **Need for alternative models**



Bewick *et al.* (2019)

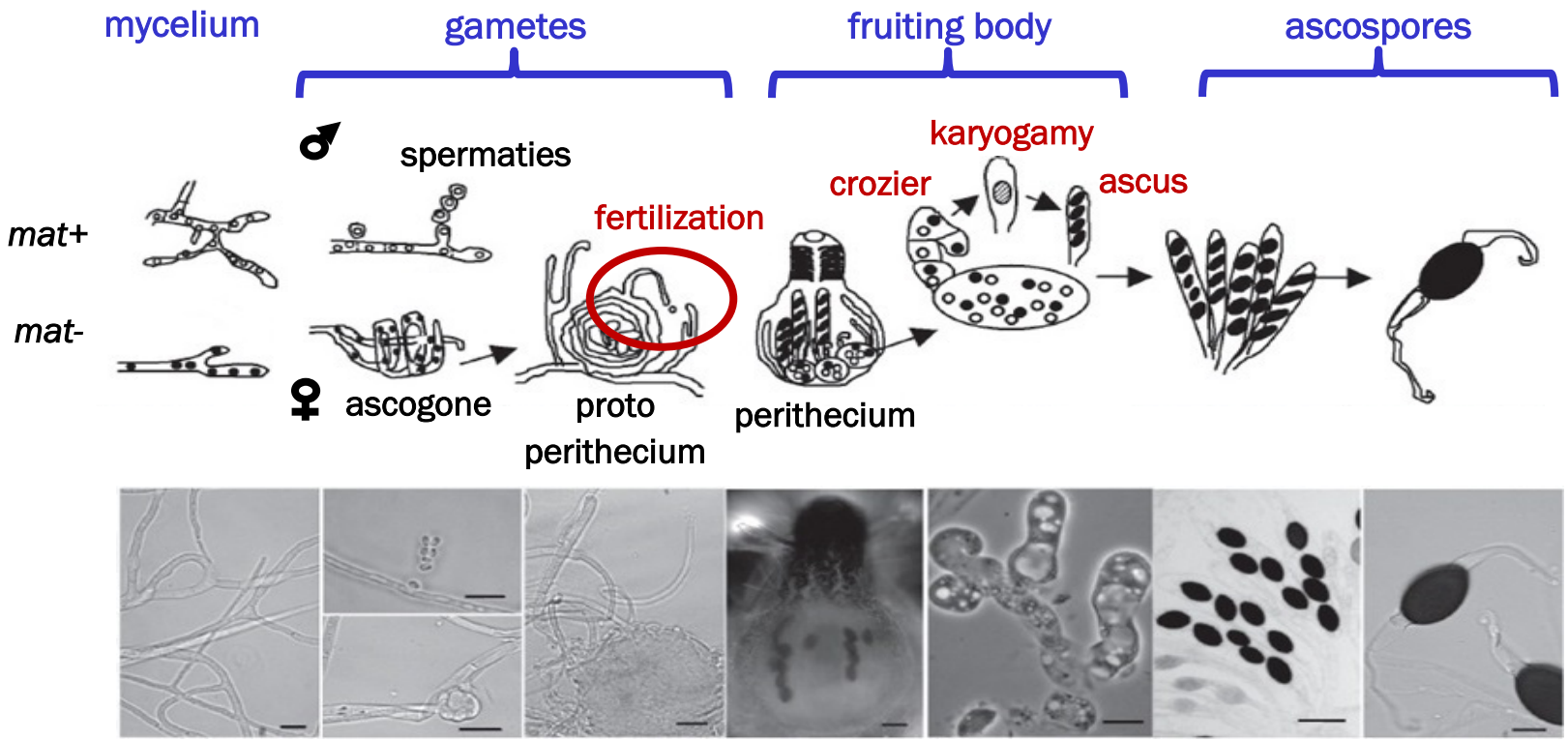
RID and sexual development

		Fertility	*de novo* DNA methylation
Neurospora crassa			
NcRID 845 aa	 *ΔRID*	+	--
Ascobolus immersus			
Masc1 537 aa	 *ΔMasc1*	--	--
Aspergillus nidulans			
DmtA 615 aa	 *ΔDmtA*	--	NA
Podospora anserina			
PaRid 752 aa	 *ΔPaRid*	-- (♀ sterile)	NA

Role of RID (and RIP) in sexual development programs ?

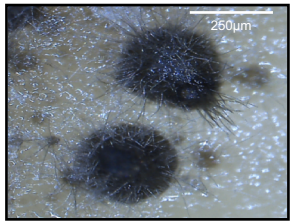
Malagnac *et al.* (1997) ; Freitag *et al.* (2002) ; Lee *et al.* (2008) ; Grognet et al. (2019)

Sexual reproduction in *P. anserina*

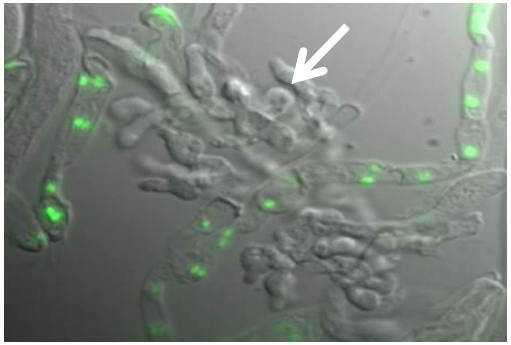


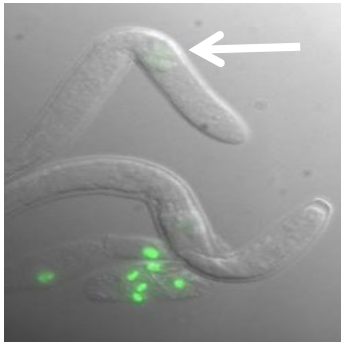
Why are Δ*PaRid* mutants sterile ?

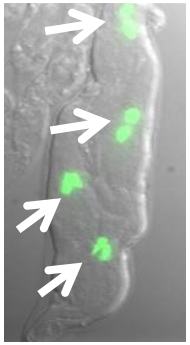
ECFG15
ROME • ITALY 2020



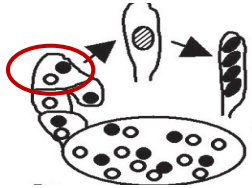
♀*PaRid+* × ♂*PaRid+*



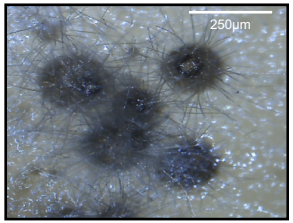




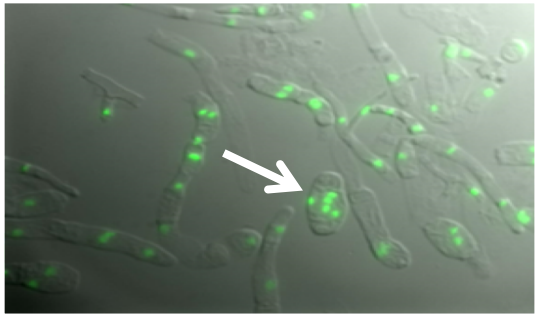
crozier



Visualization of nuclei H1-GFP



♀ Δ*PaRid* × ♂*PaRid+*



PaRid essential to the formation of dikaryotic cells

➢ When RIP happens !

Grognet *et al.* (2019)

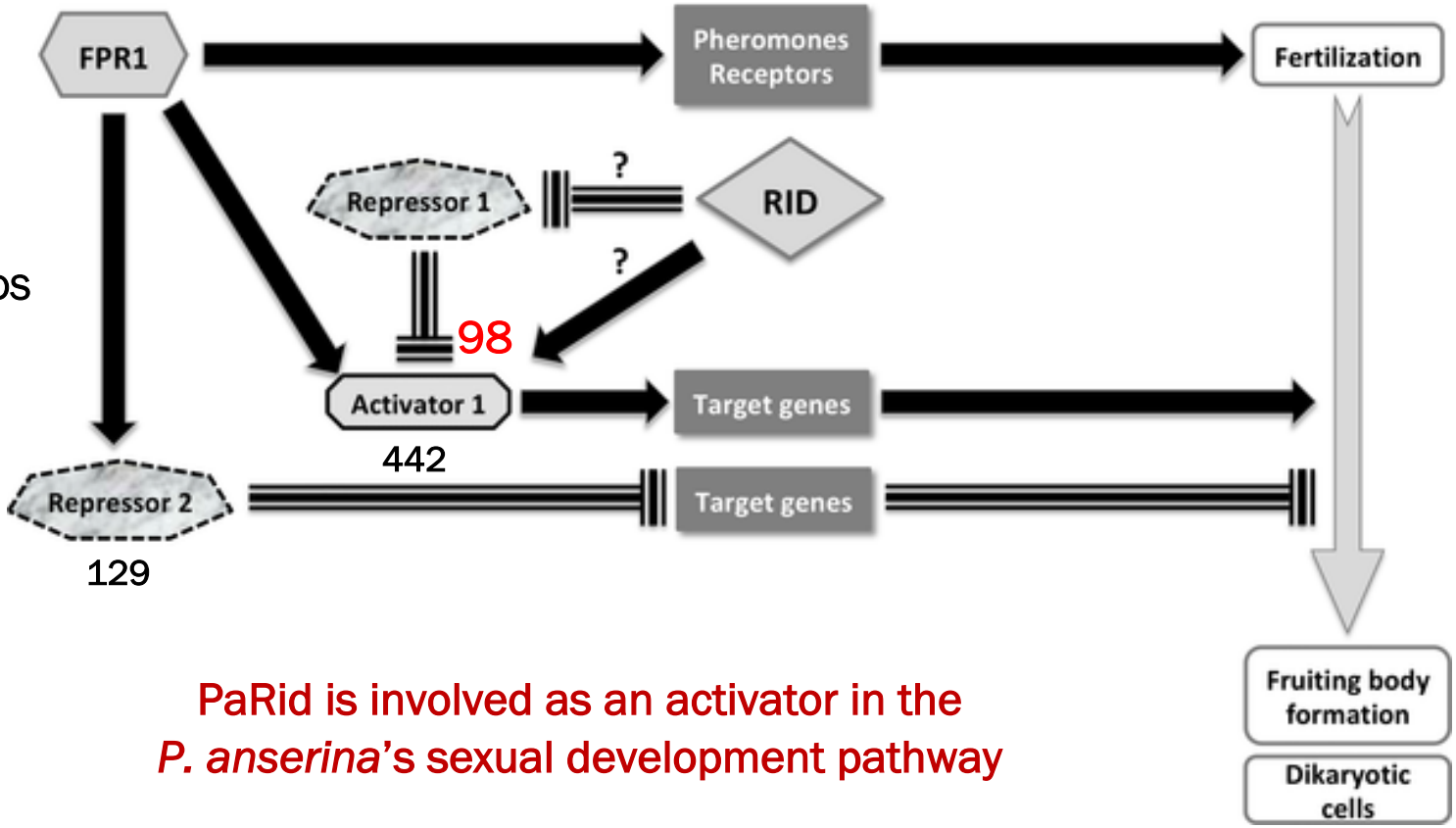
Working hypothesis

- Link(s) between RIP / RID and sexual reproduction :
  - RIP as a genome integrity check-point essential for completion of sexual reproduction ?
    - ✓ RIP effective in *P. anserina*
  - RID as an actor of an imprinting system, through *de novo* DNA methylation ?
    - ✓ No methylation detected on genomic DNA

PaRid network ?

Transcriptomic analysis
ΔPaRid vs WT

- down-regulated 217 CDS (98 shared with FPR1)
- up-regulated 234 CDS



PaRid is involved as an activator in the *P. anserina*'s sexual development pathway

Grognet *et al.* (2019)

Is the methylation activity essential ?

alleles
HA-tag

ΔPaRid + 1 ectopic allele :

Is fertility restored ?

PaRid

YES

C403S

PaRid*

NO

Site directed
mutagenesis

DNA methyltransferase activity is essential for sexual development

➢ Substrat ?

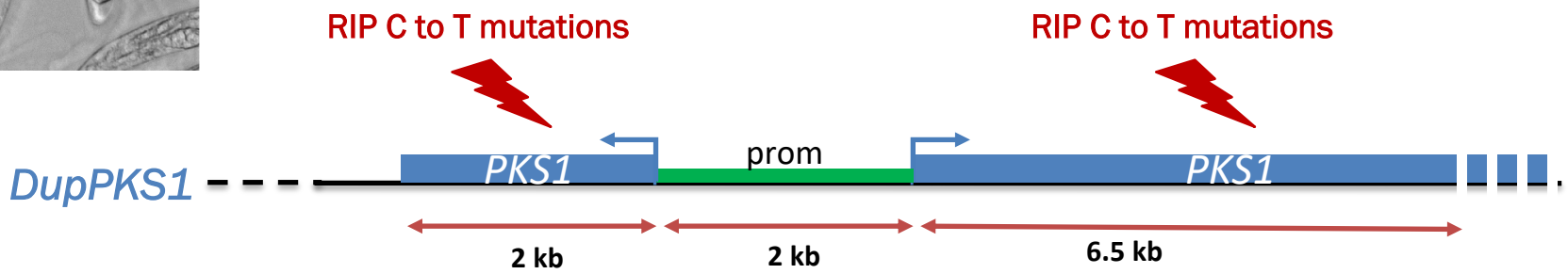
Grognet *et al.* (2019)

Is PaRid is required for RIP in *P. anserina* ?

- Construction of a RIP read-out

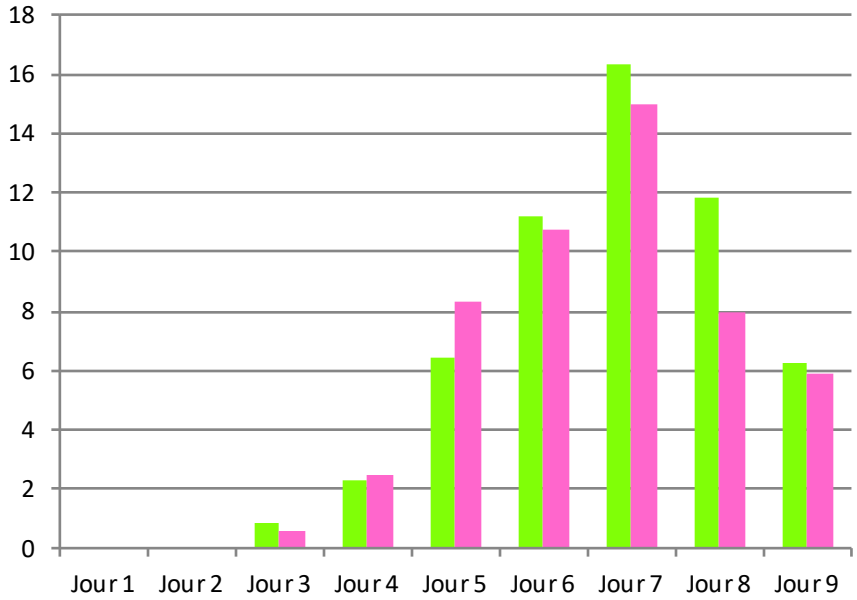
  - *PKS1* codes for the enzyme involved in the first step of melanin biosynthesis

➢ Strains harboring PKS1 duplication (*DupPKS1*) produce asci with whites spores due to RIP



Grognet and Malagnac, unpublished

RIP features in *P. anserina*



♂***PaRid DupPKS1*** x ♀***PaRid***

♂***PaRid*** x ♀***PaRid DupPKS1***

~100 meiosis / fruiting body

Days of spores production

Orientation of crosses does not affect RIP efficiency

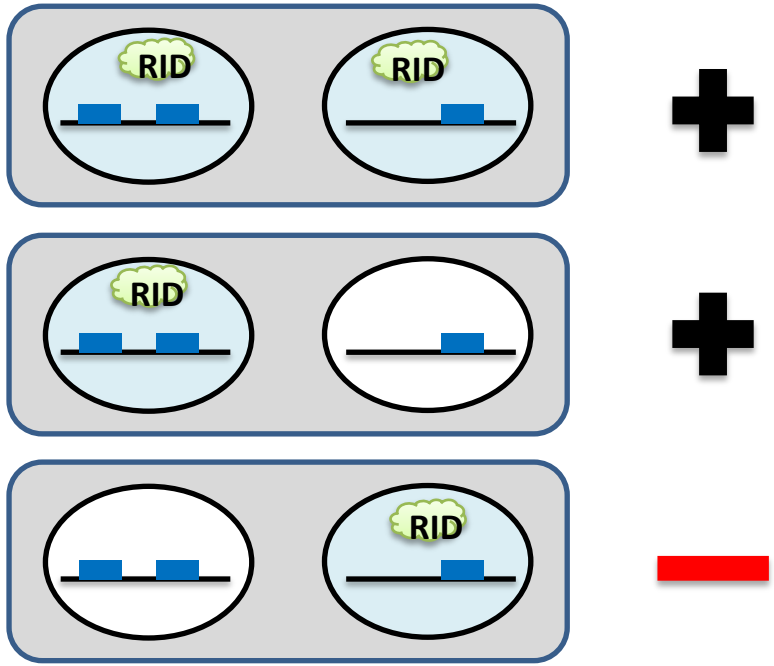
Grognet and Malagnac, unpublished

PaRid is required for RIP in *P. anserina*

PaRid DupPKS1 X *PaRid*

♀*PaRid DupPKS1* X ♂*ΔPaRid*

♂*ΔPaRid DupPKS1* X ♀*PaRid*



PaRid is essential to RIP in the haploid nucleus carrying the duplication

Grognet and Malagnac, unpublished

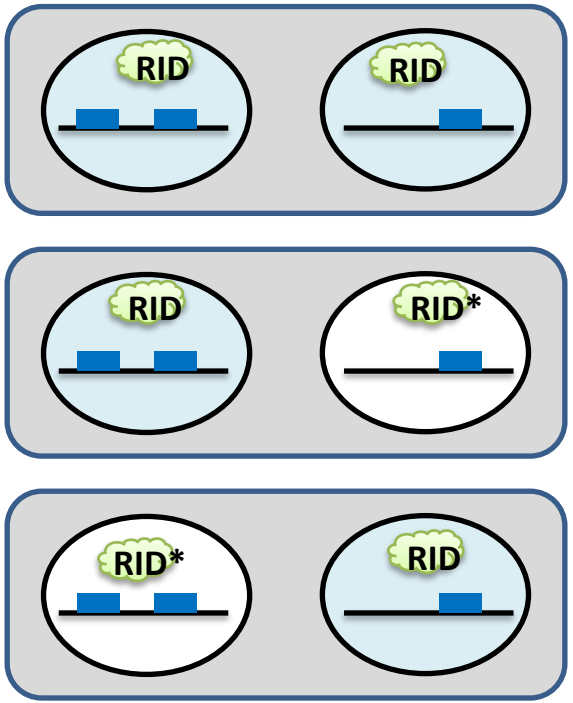
Active PaRid is required for RIP

PaRid DupPKS1 x *PaRid*

♀*PaRid DupPKS1* x ♂*PaRid**

*C403S-> ~~CH_3~~-C

♂*PaRid* DupPKS1* x ♀*PaRid*



Methyltransferase activity of PaRid is essential to RIP

Grognet and Malagnac, unpublished

Chromatin modifiers : writers and readers

Constitutive heterochromatin

DNA repeats (RIPed loci)
Subtelomeric DNA domains
Centromeres

Histone mark: H3K9me3

Writer = PaKmt1
Reader = PaHP1

Chromosome mechanism and structure
Transposable element silencing

Facultative heterochromatin

Coding sequences

Histone mark: H3K27me3
Polycomb group

Writer = PaKmt6

Gene expression regulation
Development

Chromatin features & RIP in *P. anserina*

- PaKmt1 → H3K9me3
  PaHP1 reader
  Constitutive heterochromatin

- PaKmt6 → H3K27me3
  Facultative heterochromatin

Crosses	RIP efficiency	♀ Fertility
ΔPaKmt1 X *ΔPaKmt1 DupPKS1*	+	+
ΔPaHP1 X *ΔPaHP1 DupPKS1*	+	+
ΔPaHP1 ΔPaKmt1 X *PaKmt1Δ PaHP1Δ DupPKS1*	+	+
ΔPaKmt6 X *ΔPaKmt6 DupPKS1*	-	+/-

ΔPaKmt6 defective for RIP AND affected in sexual reproduction

➢ Reminiscent of PaRid ?

Grognet and Malagnac, unpublished

Conclusions and perspectives

- ✓ *PaRid* is essential to RIP and sexual development and acts as an early activator of this developmental pathway, along with mating-type transcription factor

- ✓ 'De novo' methyltransferase catalytic activity is required for both RIP and sexual development

- ✓ PaKmt6 (H3K27me, PRC2-related complex, facultative heterochromatin) is required for proper sexual development and RIP

  - ➢ Co-factors, unknown additional function(s) of RID-like proteins ?
    - ✧ Identification of suppressors
    - ✧ KO of some of the genes differentially expressed
    - ✧ PaRid co-IP & mass spec

  - ➢ Substrat DNA or RNA ? Transient imprinting ? => maternal effect
    - ✧ PaRID ChIP-seq
    - ✧ iCLIP: Protein–RNA interactions

Acknowledgements

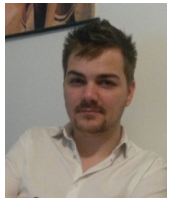
ECFG15
ROME • ITALY 2020

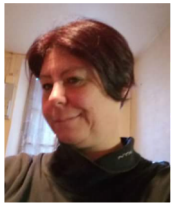
Annie Sainsard
Emeritus Professor

Fabienne Malagnac
Professor

Pierre Grognet
Assistant Professor

Florian Carlier
PhD student





Sylvie François
Laboratory Technician

CNRS
University Paris Sud
BIG LIDEX Paris Saclay
LABEX ARBRE INRA Nancy – Ascotube Project
IFPEN