RNAi in Genome Rearrangement and Chromosome Segragation in *Tetrahymena*

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1. The two features of *Tetrahymena* biology that will be emphasized are its nuclear dimorphism and the genome rearrangements that accompany development of the somatic macronucleus.

2. It is also worth mentioning that studies in *Tetrahymena* are greatly facilitated by the fact that DNA-mediated transformation occurs entirely by homologous integration and by the recent availabilty of the complete genome sequence in searchable (but not yet annotated) form.

Features of the *Tetrahymena* System

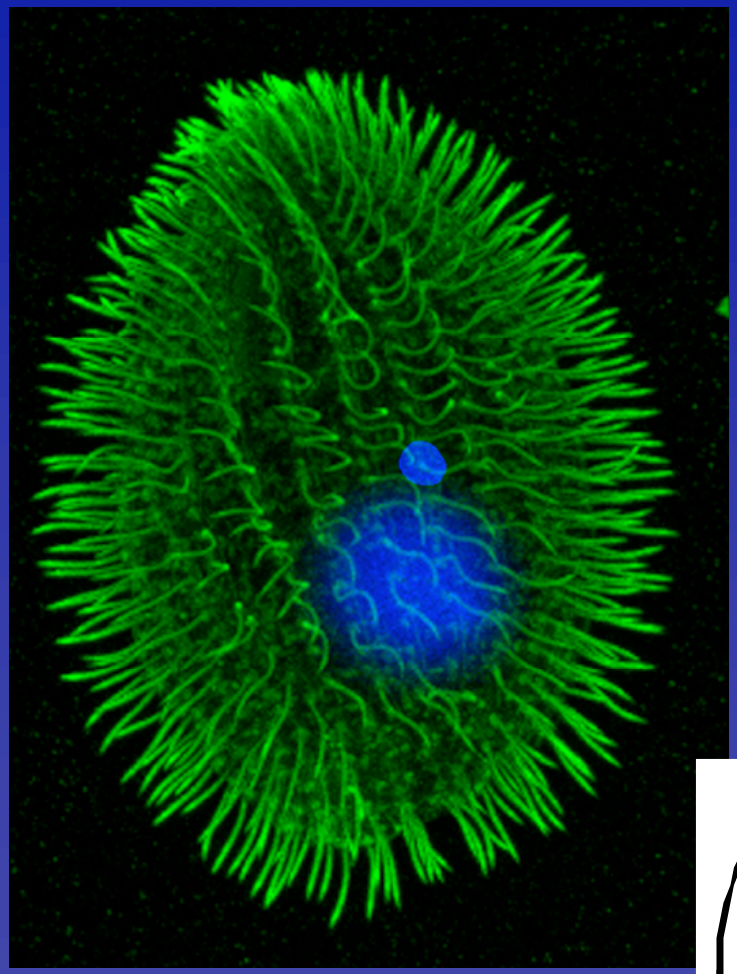
Nuclear dimorphism

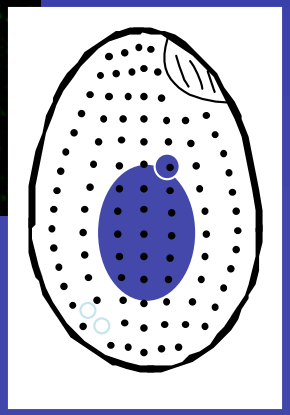
Massive genome rearrangement

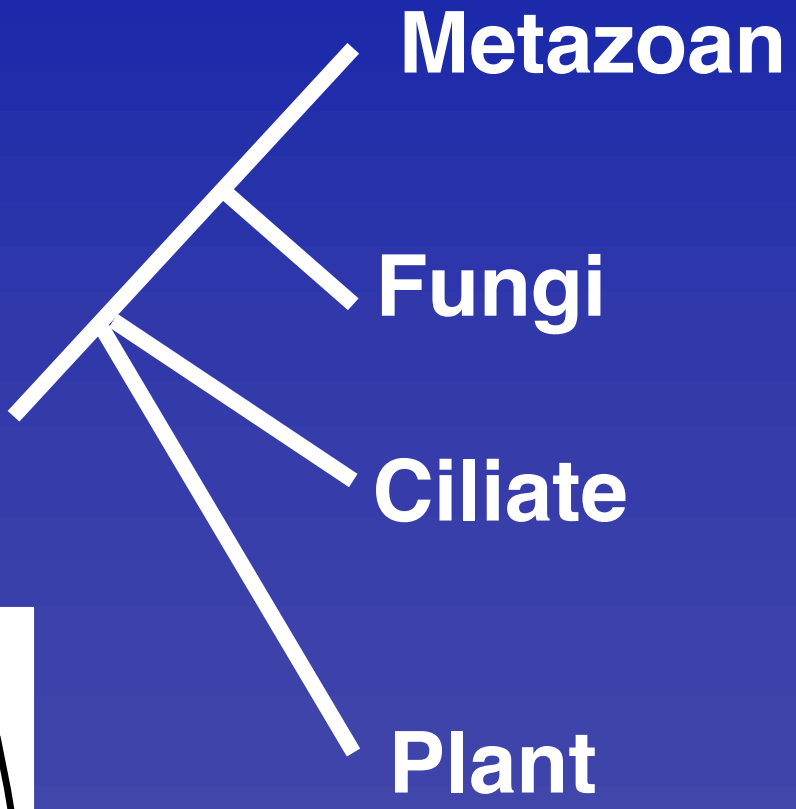
DNA-mediated transformation by homologous integration

Genome sequence (searchable, but not yet annotated)

Tetrahymena thermophila





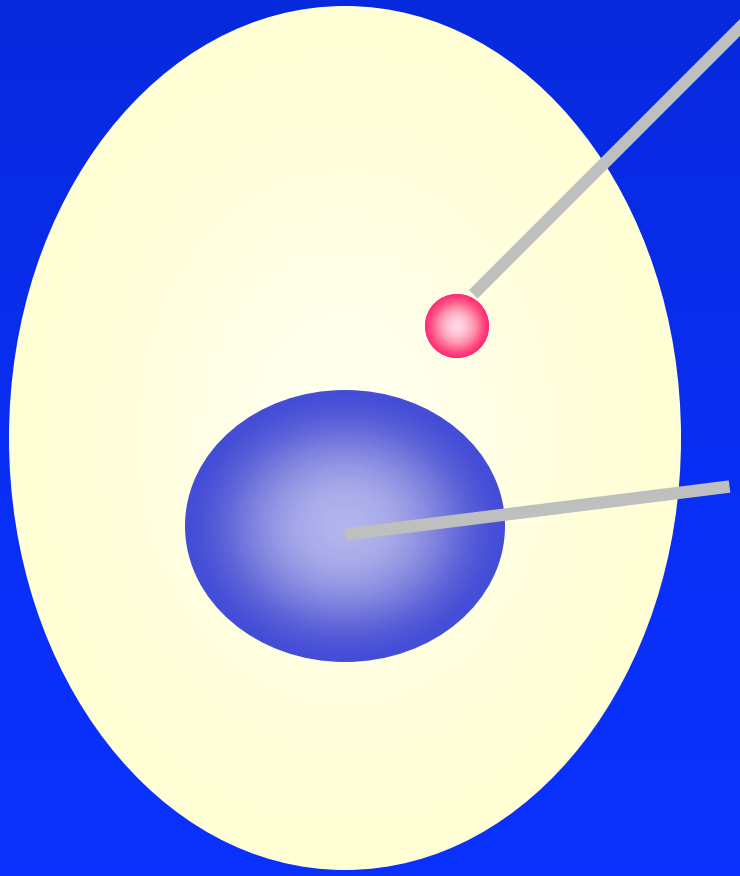


1. The 2 nuclei in *Tetrahymena* are the macronucleus (Mac) and the micronucleus (mic). Their properties are illustrated on the next slide.

2. During the sexual phase of the life cycle (conjugation) 2 cells mate and the Mic undergoes meiosis to give rise to pronuclei that are reciprocally exchanged between the 2 cells, followed by fertilization. Two post-zygotic divisions follow and the products develop into new Macs and new Mics. When the new Macs begin to develop, the old Mac becomes pycnotic, undergoes an apoptosis-like degradation and then disappears.

3. During macronuclear development, 10-15% of the micronuclear genome is eliminated.

Nuclear Dimorphism in *Tetrahymena thermophila*



Micronucleus (Mic)

- Germline
- Transcriptionally Inert (Vegetative Cells)
- Mitotic Division
- Diploid (2C); N=5

Macronucleus (Mac)

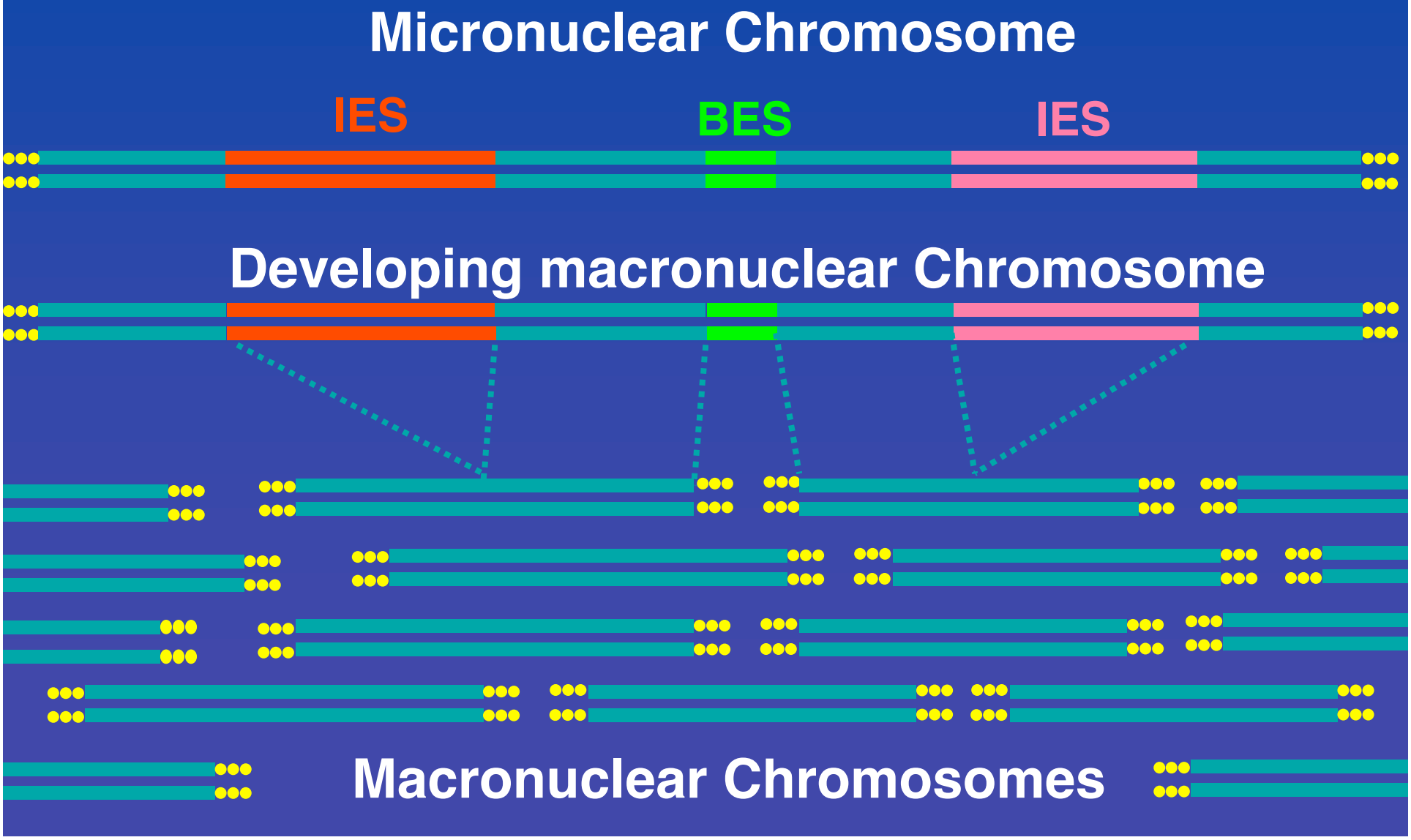
- Somatic line
- Transcriptionally Active
- Amitotic Division
- Polyploid (~45C); N=250
- Lacks 10~15% of Mic Genome

1. The next slide illustrates the 2 processes that account for DNA sequence elimination during Mac development: IES (Internal eliminated sequence) removal and chromosome fragmentation.

2. In IES removal, DNA segments ranging from 0.5-20 kb are removed and the sequences flanking them are rejoined. IES removal occurs at about 6000 sites in the macronuclear genome and is highly, but not perfectly, reproducible. IES removal accounts for most of the sequence elimination.

3. In chromosome fragmentation, breakage and elimination occurs at specific BES (breakage elimination sequence) sites. Breakage is followed by resection of ~50 bp and addition of telomeres, to form ~250 macronuclear chromosomes from the 5 micronuclear chromosomes. These then endoreplicate to produce about 45 copies each chromosome/G1 cell.

Comparison of Mac and Mic Chromosomes

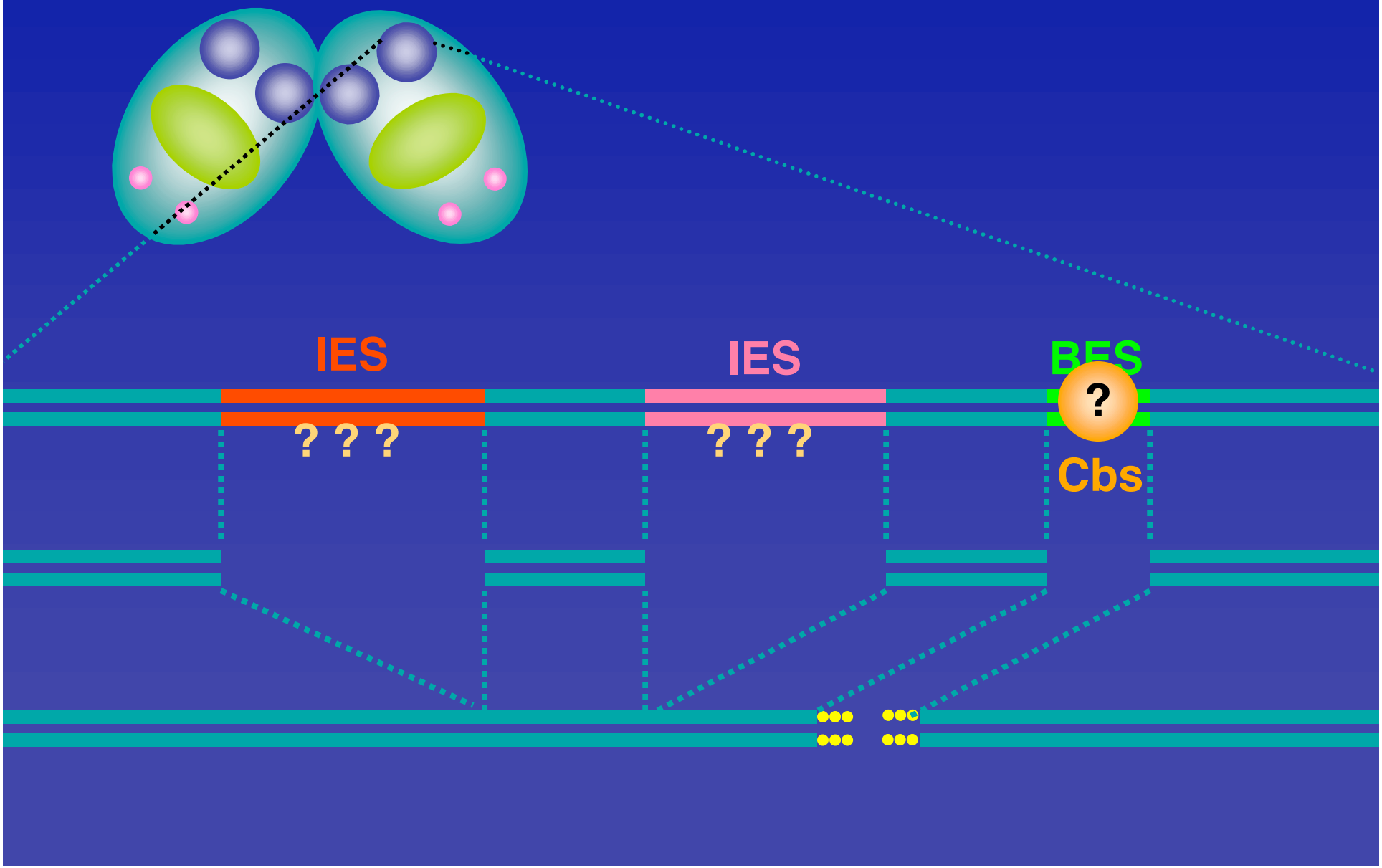


1.The next slide illustrates what is known about the mechanism of DNA elimination at IESs and BESs.

2. At BESs, a 15 bp sequence known as a Cbs (chromosome breakage sequence) has been identified by Yao and colleagues and shown to be necessary and sufficient for breakage to occur. It is assumed that a specific protein or protein complex recognizes this sequence and that a small amount of sequence elimination accompanies the process of breakage and telomere addition.

3. Comparison of the sequences of a number if IESs has failed to reveal any common sequence elements that might provide the recognition sites for their programmed elimination.

How are IES and BES recognized?



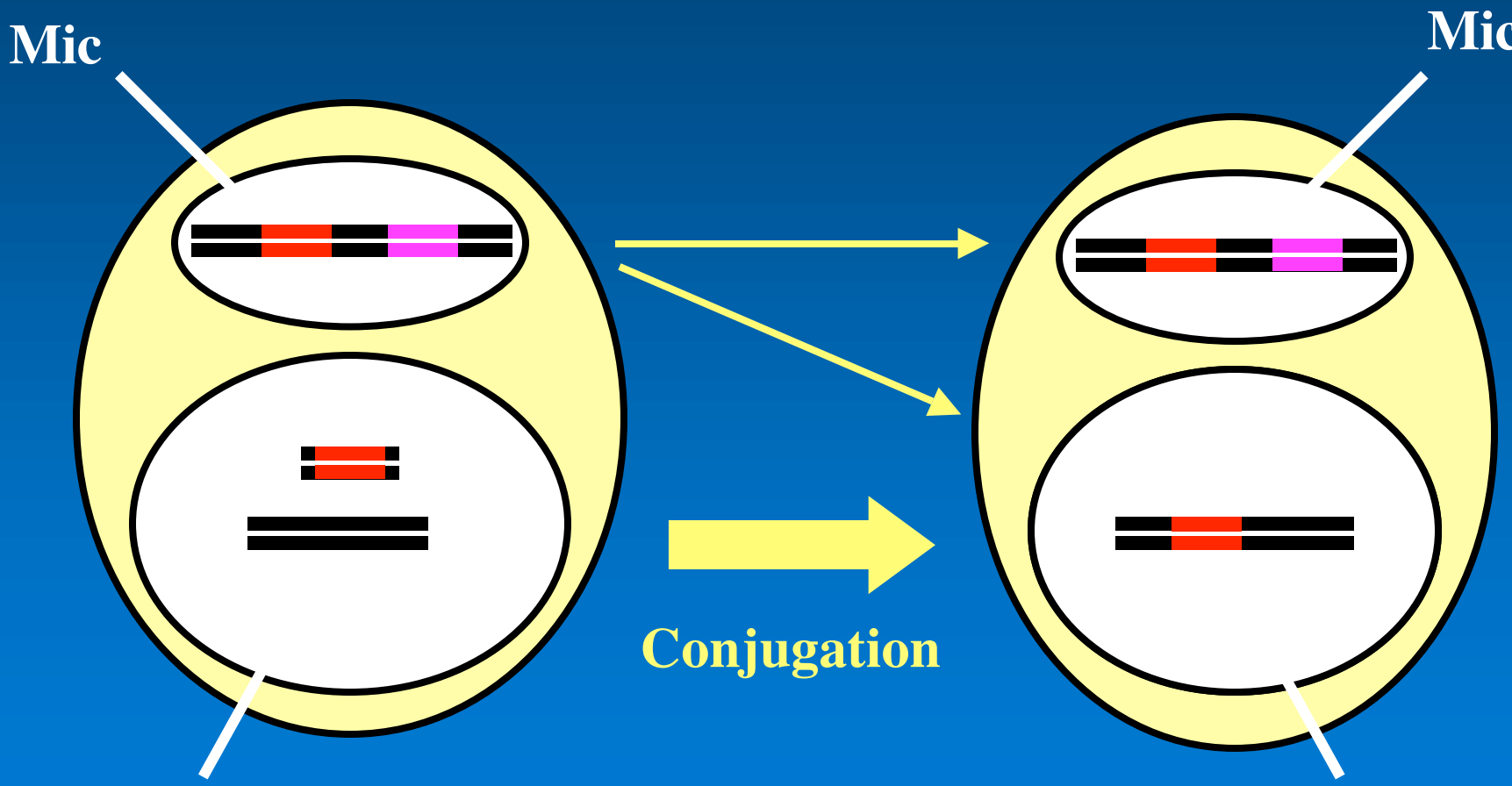
1. The next 2 slides illustrate a fascinating observation made by Chalker and Yao about IES elimination.

2. When a typical cell (shown on the left) is allowed to conjugate, the old macronucleus is eliminated and a new one forms from the micronucleus. The new macronucleus lacks IESs just like the old one did.

3. However, when an IES is placed in the old macronucleus before and the cell is allowed to conjugate, that IES fails to be eliminated from the new macronucleus although other IESs are eliminated normally. Note that the old macronucleus with the transformed episomal IES is eliminated and the IES in the new macronucleus is retained in its original chromosomal location.

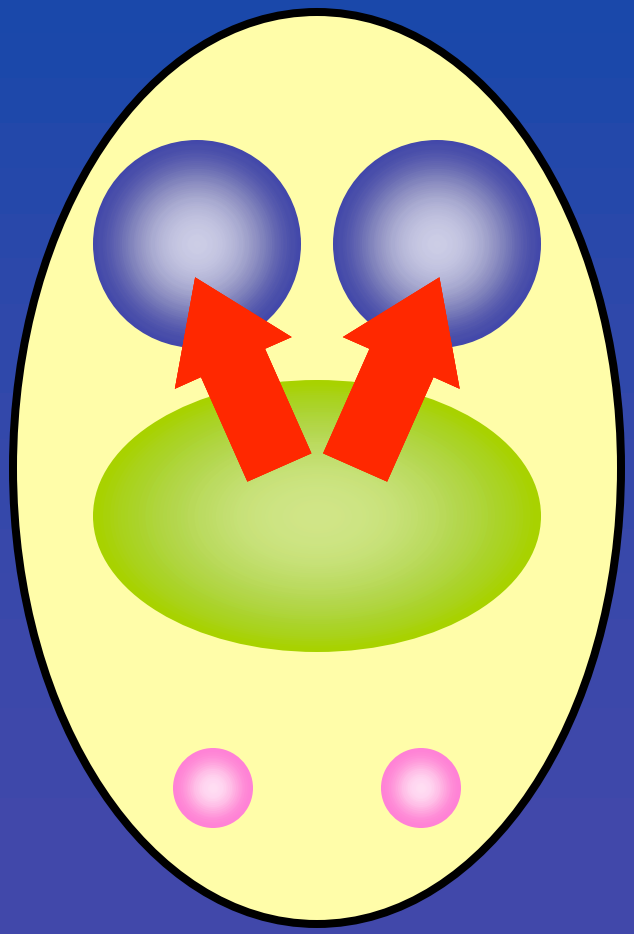
4. This experiment demonstrates an epigenetic mechanism by which sequences-specific information is transferred from the old to the new macronucleus.

Epigenetic Effect of the Parental Macronucleus on DNA Elimination



Chalker and Yao, 1996

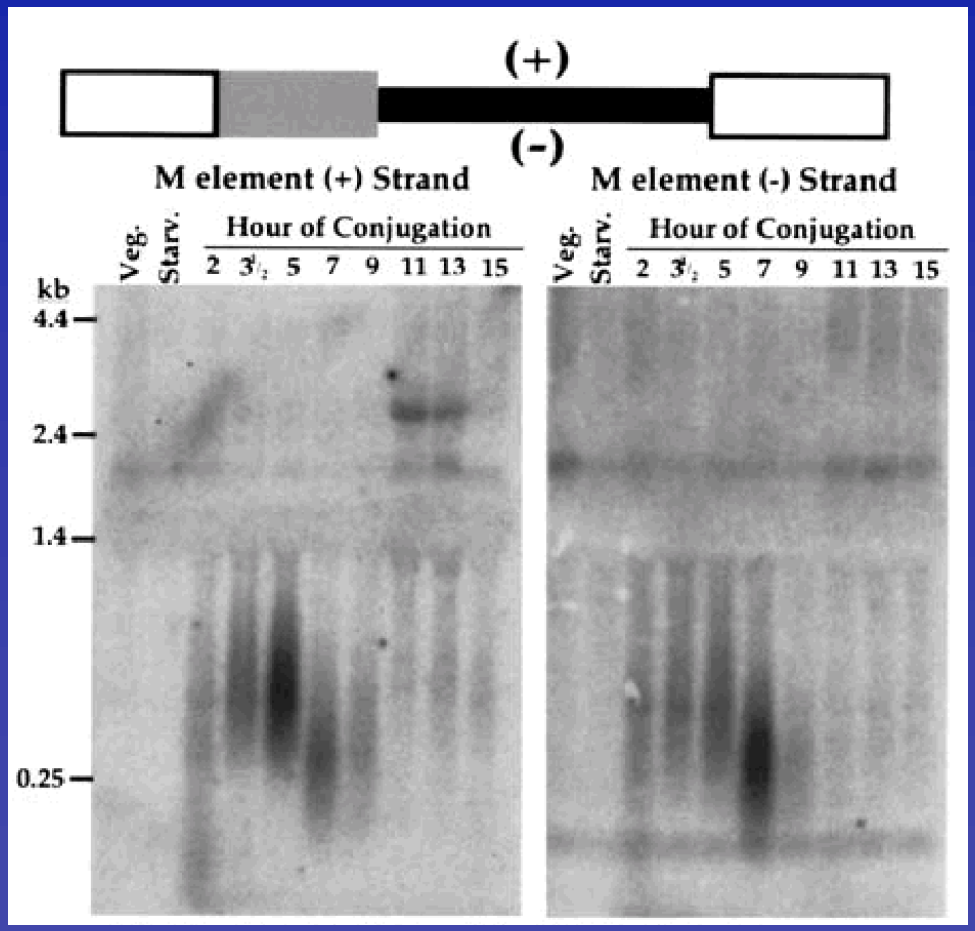
Sequence Specific Information is Transferred from the Old to New Mac



1. The next slide illustrates another observation made by Chalker and Yao. They demonstrated that transcripts containing IES sequences could be detected during early conjugation. These transcripts were heterogeneous and were derived from both strands of the IESs.

2. These observations suggest that transcripts capable of forming double stranded (ds) RNAs are synthesized by micronuclei in early conjugation.

Micronuclear Bidirectional Transcription of IESs Precedes DNA Rearrangement



Chalker and Yao (2001) *Genes Dev* 15, 1287-1298

1. The next 2 slides illustrate the organization of 8 piwi-like (*TWI*) genes in the *Tetrahymena* genome and the expression of one of them.

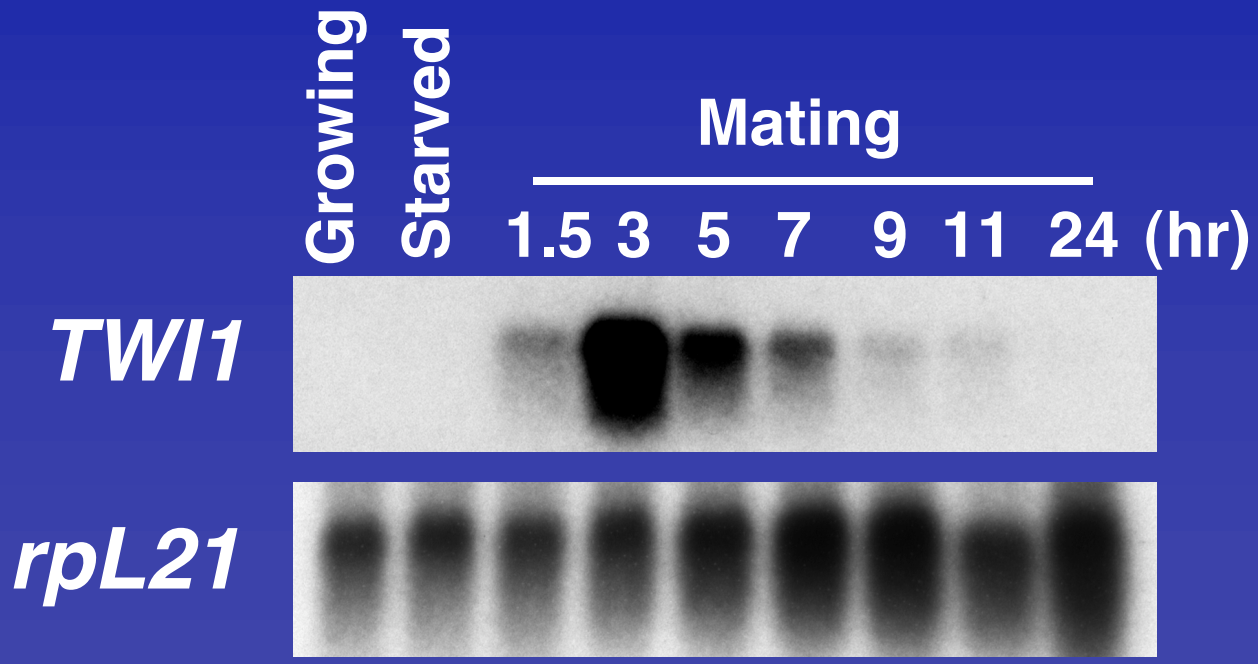
2. Piwi genes are PPD proteins, widely distributed in eukaryotes and found to be associated with RNAi processes.

3. Only the *TWI1* gene will be discussed in this presentation. Northern blot analyses indicate it is expressed only during early conjugation.

4. As expected from the fact that it is not expressed in vegetative cells, knocking out the *TWI1* gene has no effect on growth. However, cells lacking *TWI1* genes in their macronuclei fail to yield any progeny when they conjugate.

There are 8 piwi-related *TWI* Genes in *Tetrahymena*

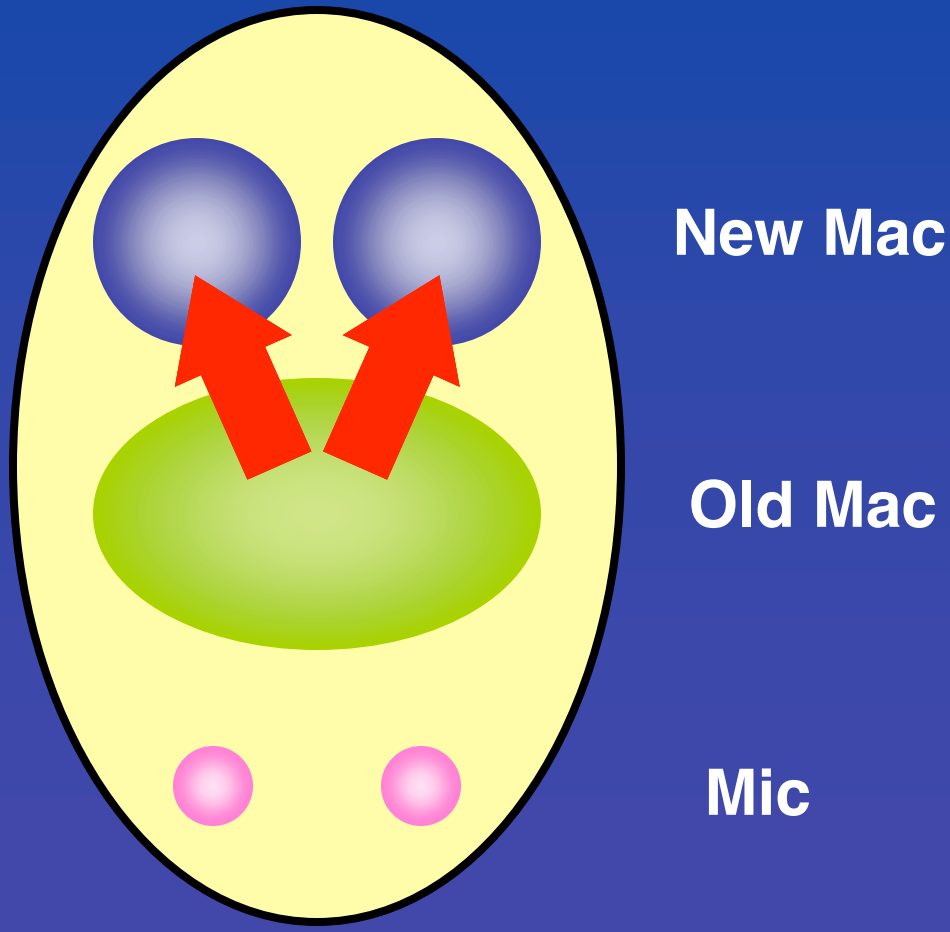
TWI1 mRNA is the Only Member of the TWI Family Specifically Expressed Only in Early Conjugation



1. The next slide illustrates (without data) an interesting property of the protein (Twi1p) encoded by the *TWI1* gene. A tagged Twi1p that can rescue the conjugation lethal phenotype of a *TWI1* deletion localizes first in the cytoplasm of conjugating cells. It then localizes exclusively in the old macronucleus. However, when the new macronucleus forms, the Twi1p localized in the old Mac is rapidly transferred to it. Twi1p disappears completely in late conjugation.

2. The transfer of the Twi1p from the old to the new Mac parallels the epigenetic transfer of sequence information observed by Chalker and Yao.

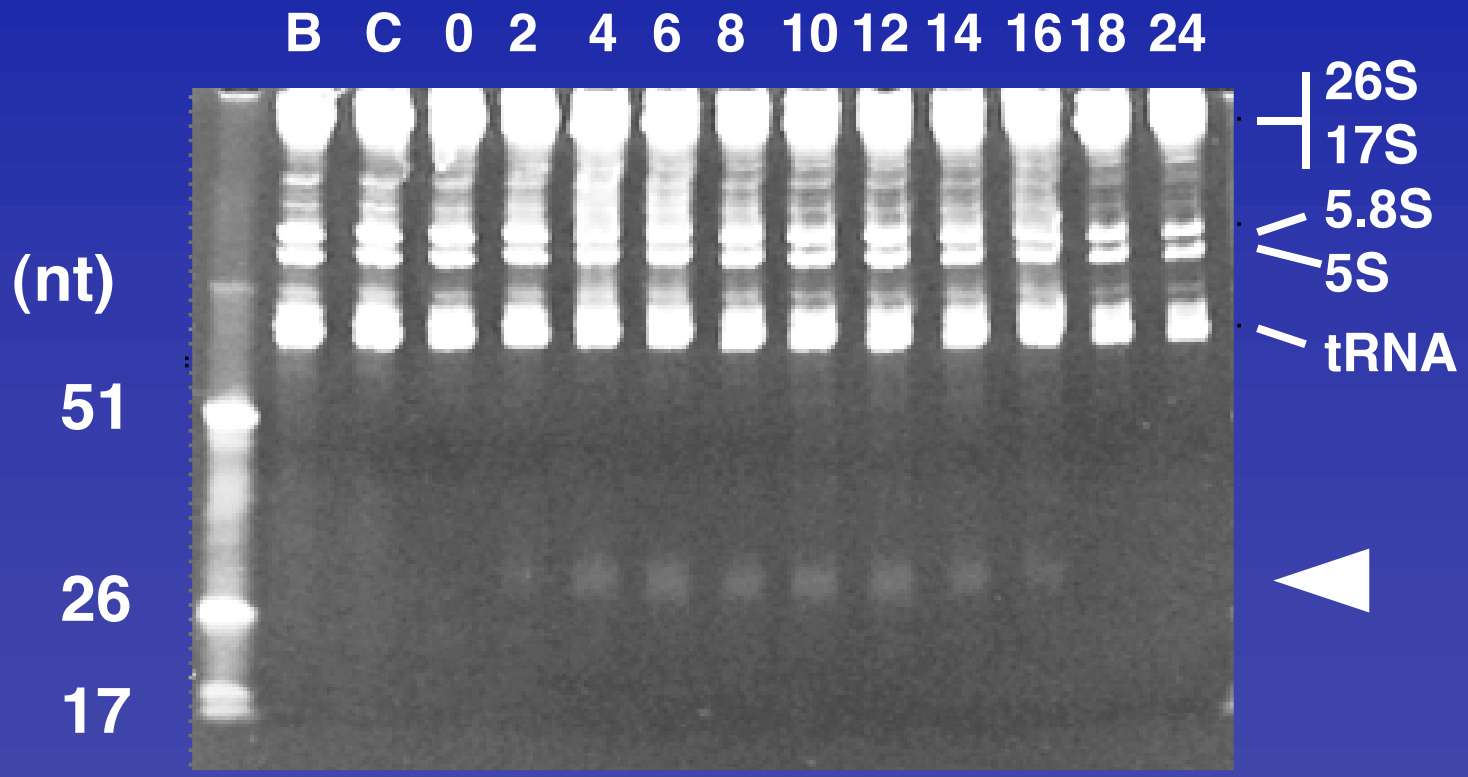
Twi1p Localizes Initially in Cytoplasm, then in the Old Mac, but is Transferred to the New Mac as Soon as it Forms



1. The next slide shows that significant amounts of small, 28 nt RNAs can be detected in conjugating cells. It appears between 0 and 2 hr after conjugation is initiated and disappears after 18 hr. These 28 nt RNAs are not detectable in vegetative cells (B, C).

2. The 28 nt RNAs appear but do not accumulate in cells in which the *TWI1* gene has been knocked out (data not shown).

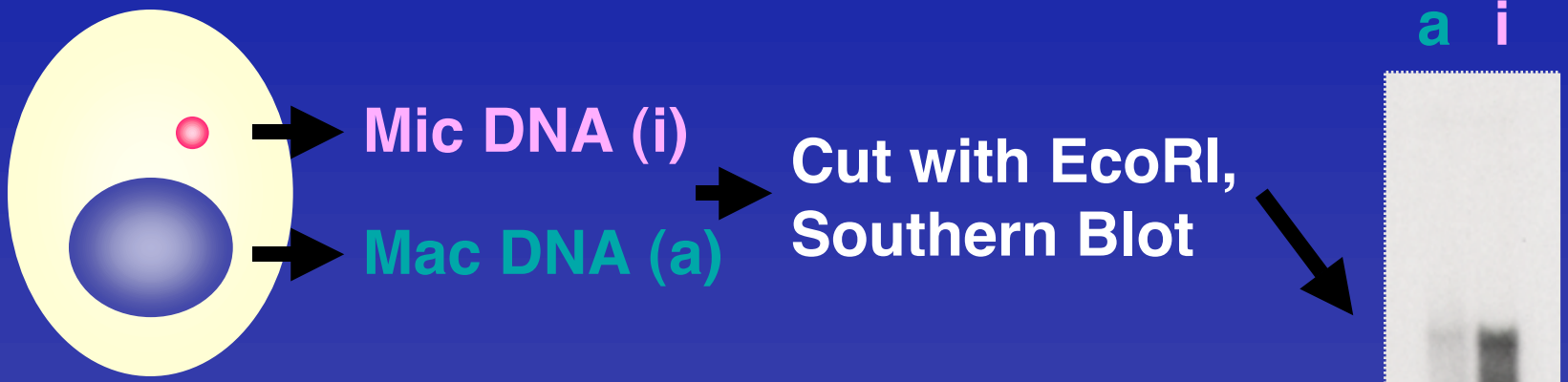
Small (28 nt) RNAs are Specifically Expressed During Conjugation

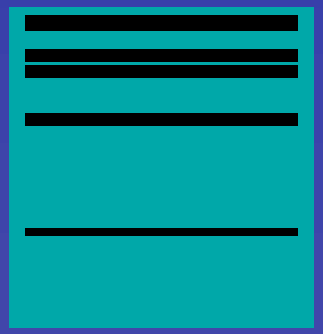


1. The next slide illustrates an experiment that establishes that a significant fraction of the 28 nt RNAs are derived from IES sequences. 28 nt RNAs were isolated and gel purified from 12 hr conjugating cells, end-labeled with ^{32}P and used as a probe to hybridize to macronuclear (a) or micronuclear (i) DNA on a Southern blot.

2. The 28 nt RNAs hybridize much more strongly to micronuclear than to macronuclear DNA and the hybridization is highly heterogeneous. These observations suggest that the 28 nt RNAs are enriched in sequences related to IESs.

Conjugation-Specific Small (28 nt) RNAs are Enriched in Micronucleus-Specific (IES) Sequences





RNA from 12h post-mixing

Extract Small RNA From Gel

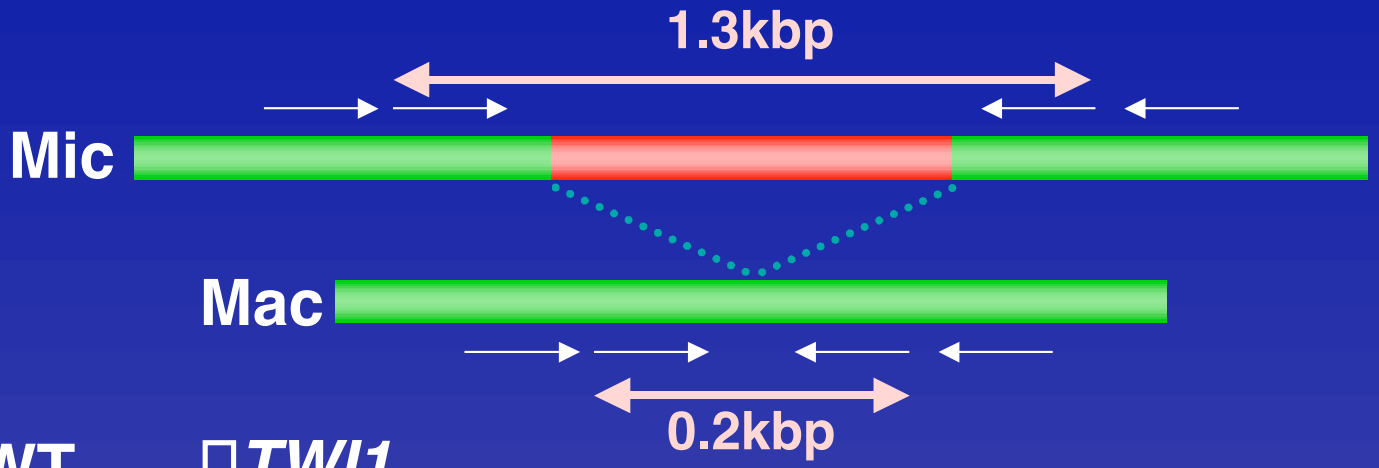
End Labeling

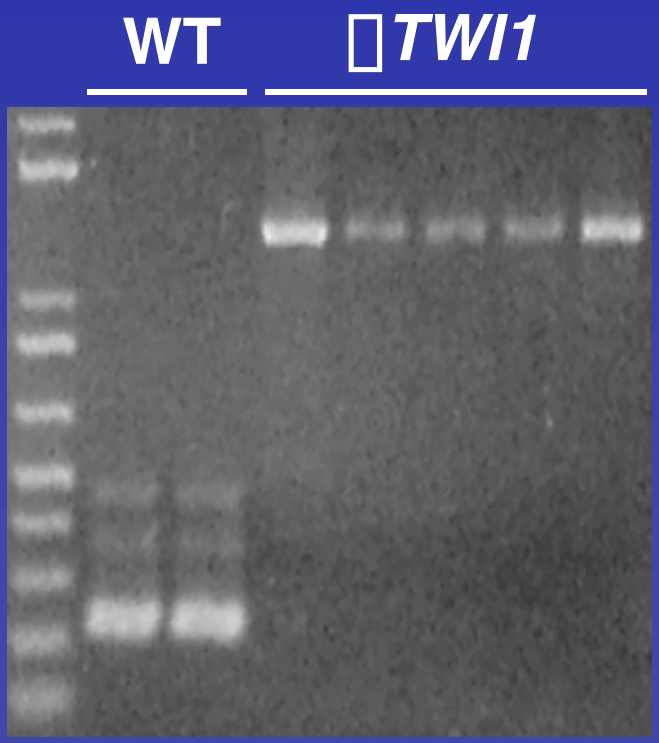
1. The next slide illustrates that the *TWI1* gene is required for IES elimination.

2. The diagram illustrates the single-cell nested PCR assay for elimination a specific, well-characterized IES (the R element). If the IES has been removed, a small (0.2 kb) fragment is expected. If the IES has been retained, a larger (1.3 kb) fragment should be observed.

3. The agarose gel demonstrates that when wild-type cells (WT) are mated, the R IES is eliminated. When *TWI1* knockout cells (□*TWI1*) cells are mated, the R element is retained.

TWI1 is Required for IES Elimination in the R-region





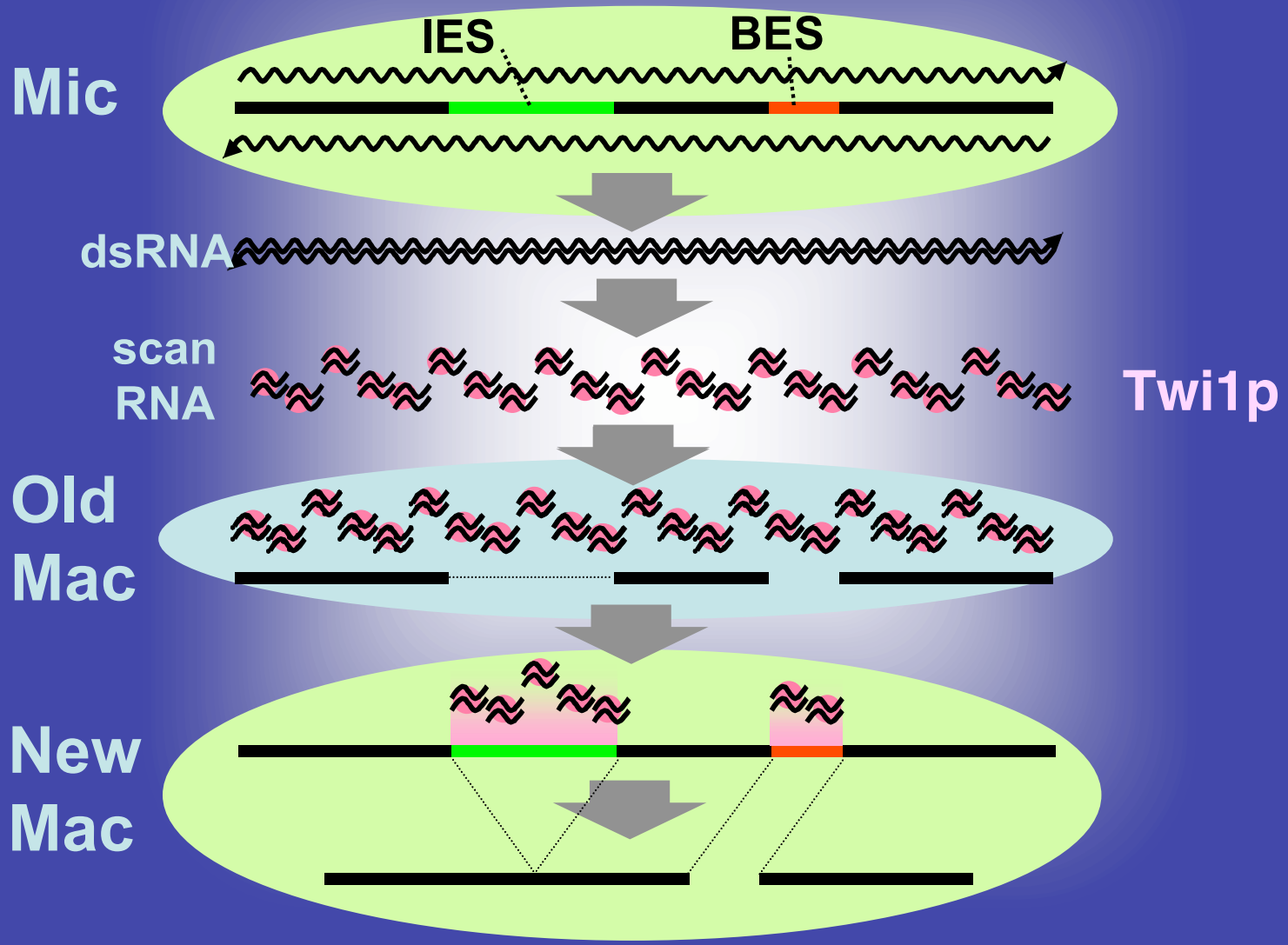
◄ Mic Form (1.3kbp)

◄ Mac Form (0.2kbp)

The next slide illustrates a model, referred to as the scan (scn) RNA model, that explains the preceding observations. It proposes the following steps in the process of IES elimination

1. dsRNAs are synthesized during early conjugation in the micronucleus and transferred to the cytoplasm.
2. The dsRNAs are cleaved by a dicer-like enzyme to 28 nt scnRNAs and associate with Twi1p.
3. scnRNAs, in association with Twi1p are imported into the old macronucleus.
4. scnRNAs "scan" the macronuclear genome. If they find a homologous sequence, they are destroyed by an unknown mechanism.
5. ScnRNAs that are not destroyed are transferred from the old to the developing macronucleus where they target IESs and BESs for elimination.

scan RNA model



1.The next slide lists 4 predictions of the scnRNA model that were tested and found to be correct.

2. Data supporting these conclusions were presented at the meeting. However, these data are unpublished and making this presentation electronically available would preclude their publication in many journals. Therefore the data are not presented here

Predictions of the scan RNA Model for DNA Elimination in *Tetrahymena*

1. **The scanning process should increase the specificity of scnRNAs for micronucelar-specific sequences as conjugation proceeds.**

2. **scnRNAs should be complexed with Twi1p.**

3. **scnRNAs should localize with Twi1p in both old and new macronuclei.**

4. **A foreign sequence present in the micronucleus but not in the macronucleus should behave like an IES.**

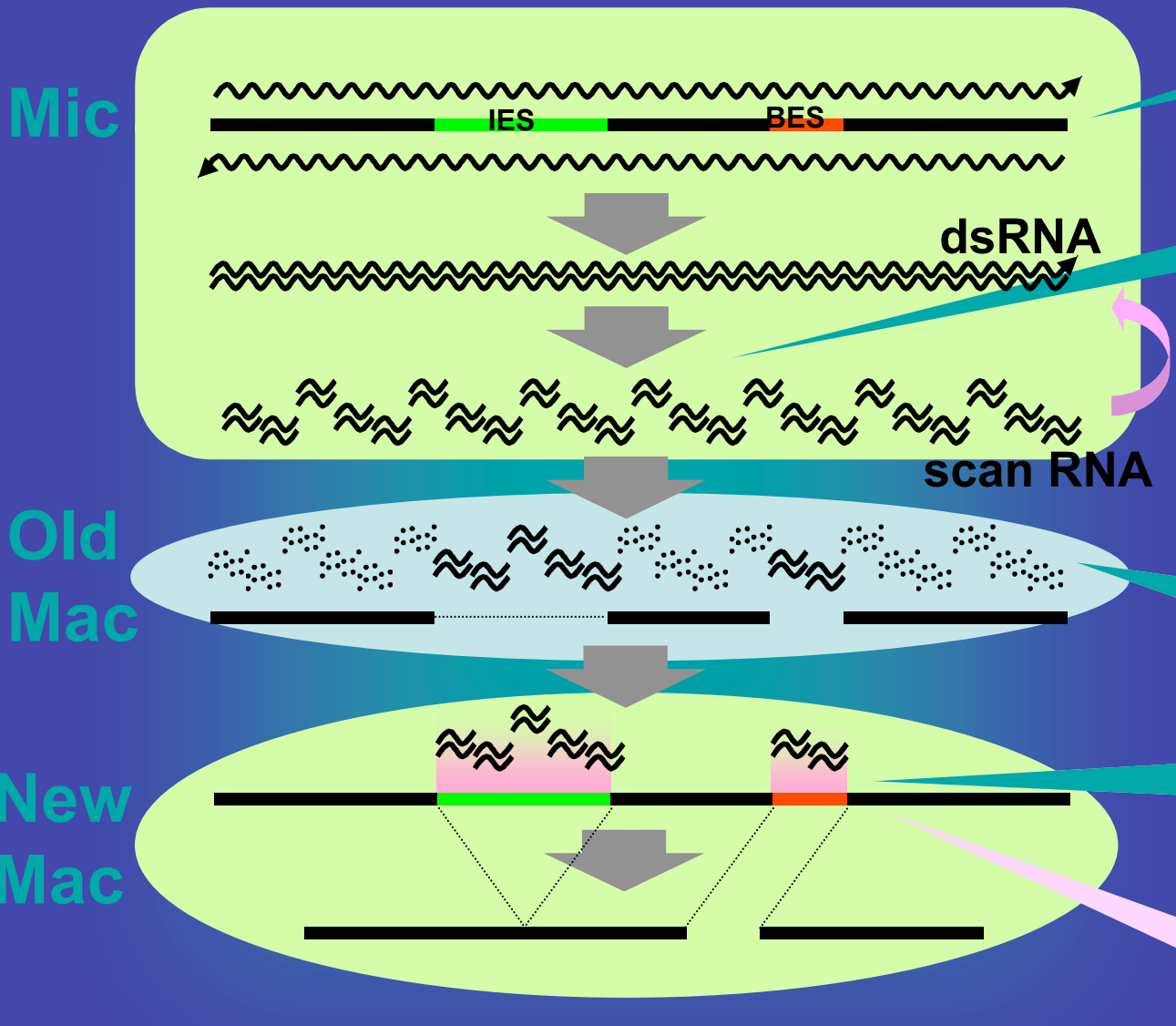
1.The next slide describes the enzymatic machineries that are likely to be involved in IES elimination by the scnRNA mechanism.

2. Evidence demonstrating the nature of the RNA polymerase and the RNAse III (dicer) were presented at the meeting. However, these data are unpublished and making this presentation electronically available would preclude their publication in many journals. Therefore the data are not presented here.

3. Evidence was presented that the actual cleavage of dsRNA to scnRNAs occurred in the micronucleus. However, these data are unpublished and making this presentation electronically available would preclude their publication in many journals. Therefore the data are not presented here.

4. Additional evidence was presented that the same dicer-like enzyme involved in IES elimination is involved in mitotic chromosome segregation in vegetative cells. However, these data are unpublished and making this presentation electronically available would preclude their publication in many journals. Therefore the data are not presented here.

Enzymatic Machineries Likely to be Involved in DNA Rearrangement



DNA dependent RNA polymerase

RNaseIII (Dicer)

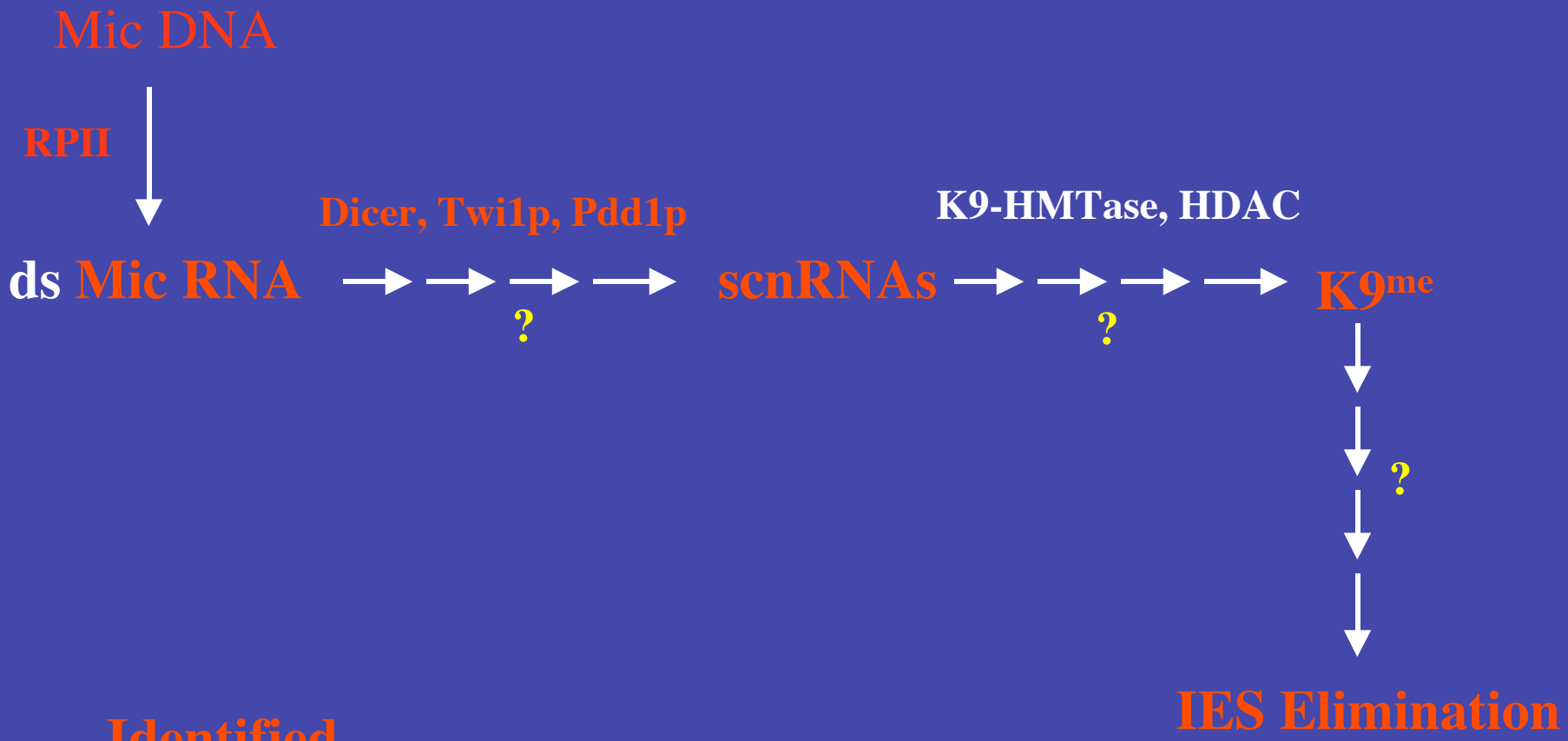
RNA dependent RNA polymerase ?

RNaseH ?

histone deacetylase

H3K9 methyltransferase

The Pathway from Mic DNA to IES Elimination



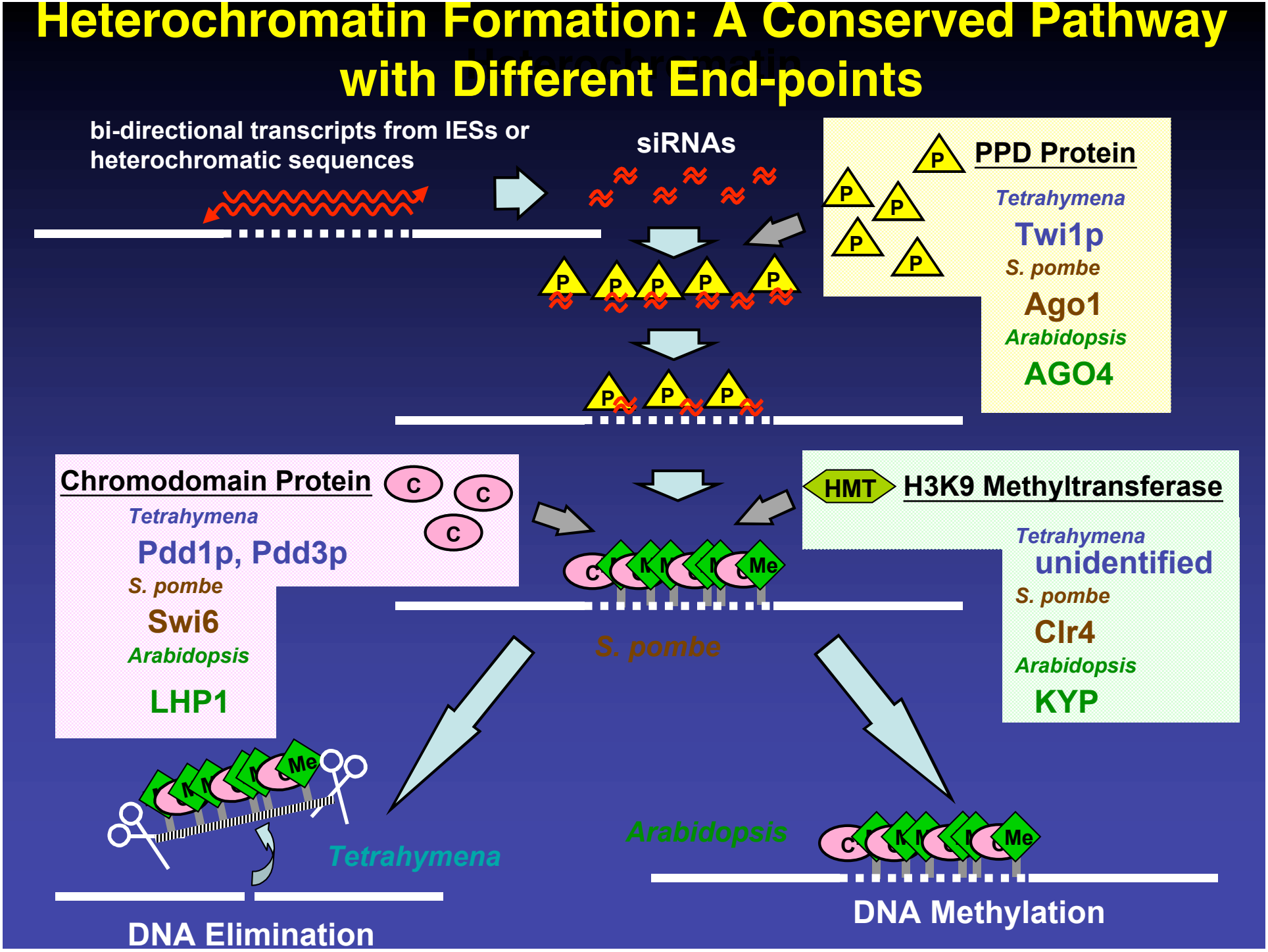
Identified
Inferred
Unknown

IES Processing in *Tetrahymena* is Remarkably Similar to Heterochromatin Silencing by *Schizosaccharomyces pombe* Centromeric Repeats[1]

Centromeric Silencing	IES processing
•Mostly repeated sequences	• Mostly repeated sequences
•Both strands are transcribed	• Both strands are transcribed
•RNAi-like mechanism •siRNA homologous to repeats •Requires RNAi genes	• RNAi-like mechanism •siRNA homologous to repeats •Requires *TWI1*
•Requires HDAC Activity	•Requires HDAC Activity
•Requires H3-K9 methylation	•Requires H3-K9 methylation
•Requires Chromodomain Proteins (HP1, Swi6p)	•Requires Chromodomain Proteins (Pdd1p, Pdd3p)

[1]Volpe TA, et. al. Science, 297: 1833-1837. 2002.

Heterochromatin Formation: A Conserved Pathway with Different End-points



Summary and Conclusions

1. **DNA elimination occurs by an RNAi-mediated process that is remarkably similar to heterochromatin formation/gene silencing in other organisms.**

2. **DNA elimination (and heterochromatin formation) probably arose as a mechanism for eliminating/silencing foreign DNAs that invade the genome.**

3. **DNA elimination involves "scanning", a novel, epigenetic mechanism that ensures only foreign sequences are eliminated.**

4. **Centromere function and DNA elimination share a common RNAi component (Dcl1p) but probably use different PPD (Twi1p-like) proteins.**

5. **There are probably additional RNAi-based processes in *Tetrahymena* (2 additional dicers 7 more TWIs awaiting functional analyses).**