

PacBio sequencing uncovers a new epigenetic mark in early diverged fungi

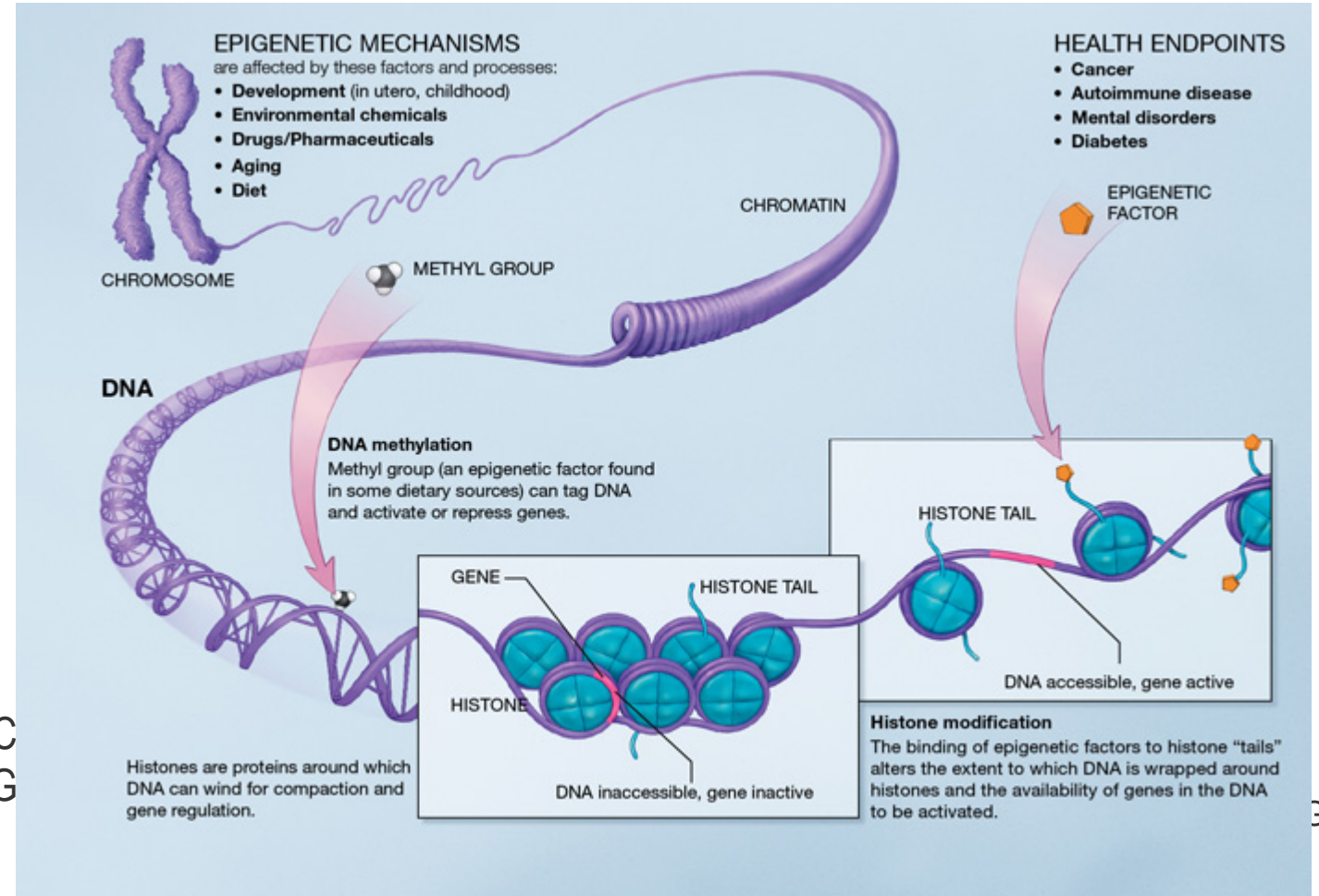
JGI User Meeting 2016

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Epigenetics and DNA-methylation

- **Epigenetics:** modification of gene expression rather than alteration of the genetic code itself.
- **5mC:** the best known DNA methylation mark
 - Done with bisulfite-seq (available at JGI)
- **5mC is:**
- **Symmetric CG**
- **Excluded from promoters typically**
- **When present, gene is repressed**

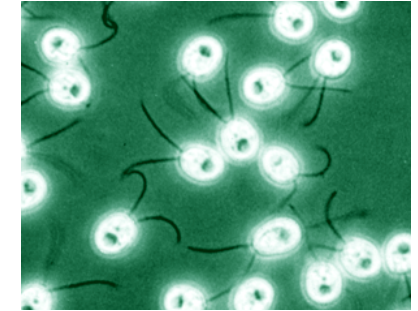
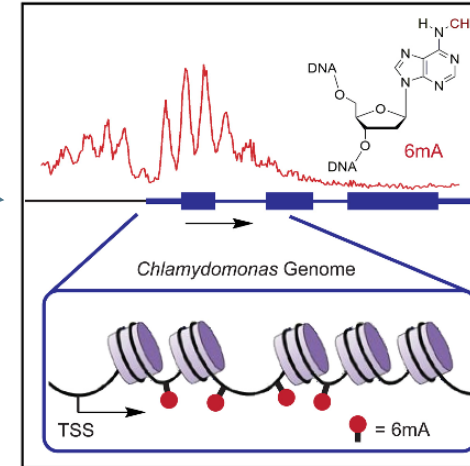
5'
AC
TG



A-methylation in eukaryotes

- ***Chlamydomonas*** – Fu et al., 2015 (Cell)
 - Bimodal distribution surrounding TSS
 - Present in 84% of genes
 - Correlated with active gene expression
 - Methods: 6mA-IP-seq, 6mA-CLIP-exo & 6mA-RE-seq
- ***Drosophila*** – Zhang et al., 2015 (Cell)
 - Methods: UHPLC-MRM-MS/MS, immunostaining/dot blot
- ***Caenorhabditis (C. elegans)*** – Greer et al., 2015 (Cell)
 - Methods: PacBio, UHPLC-MS/MS, immunostaining/dot blot
- What about fungi??

Chlamydomonas

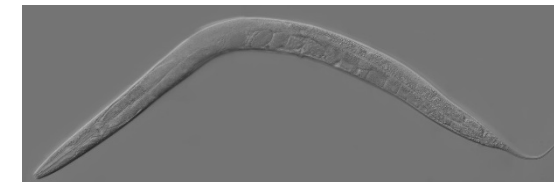


<http://genome.jgi.doe.gov/Chlre3>



Drosophila

<https://en.wikipedia.org>

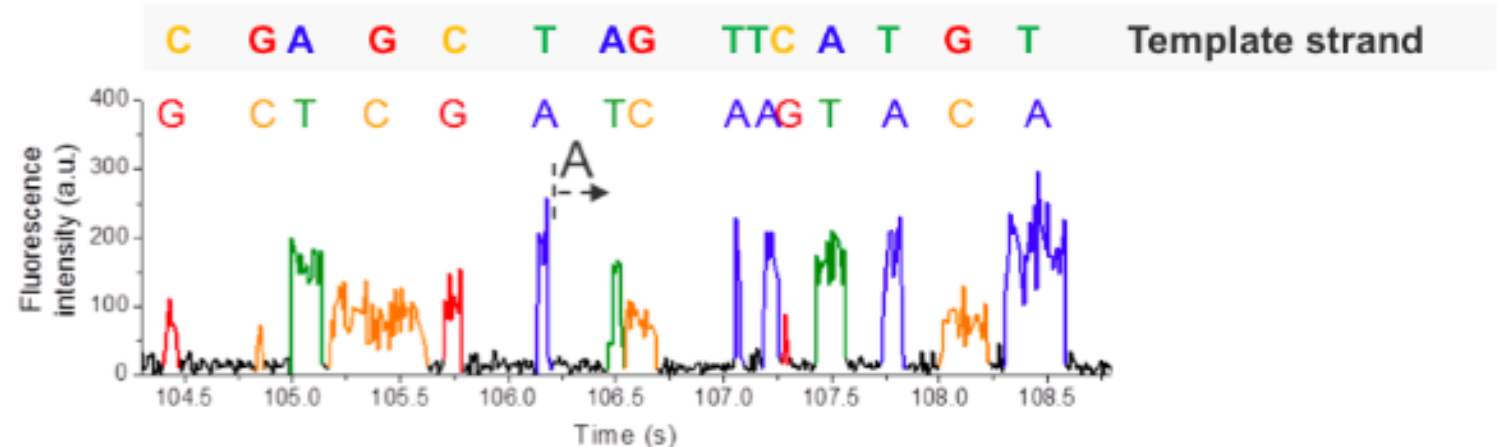
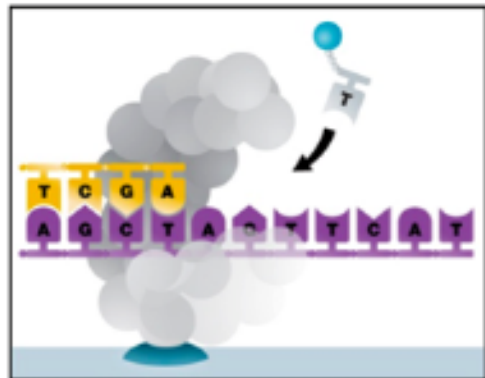
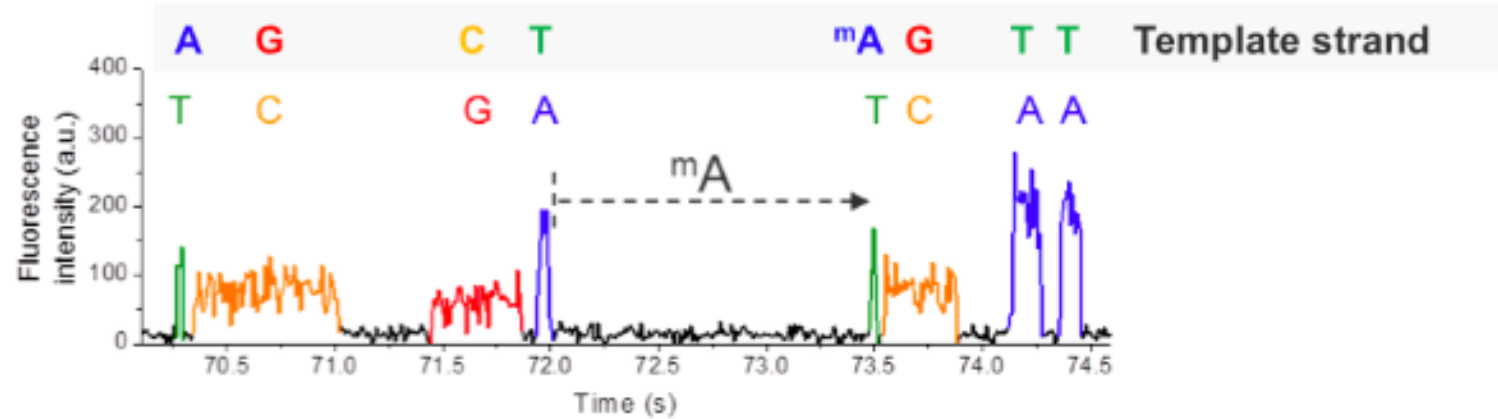
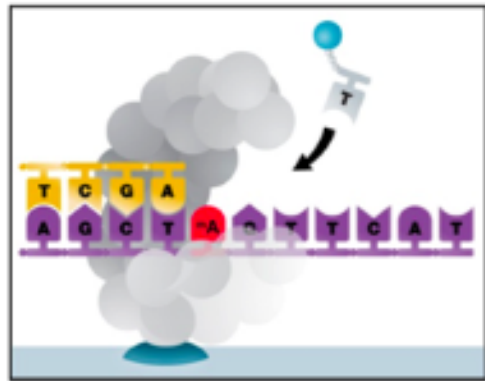


C. elegans

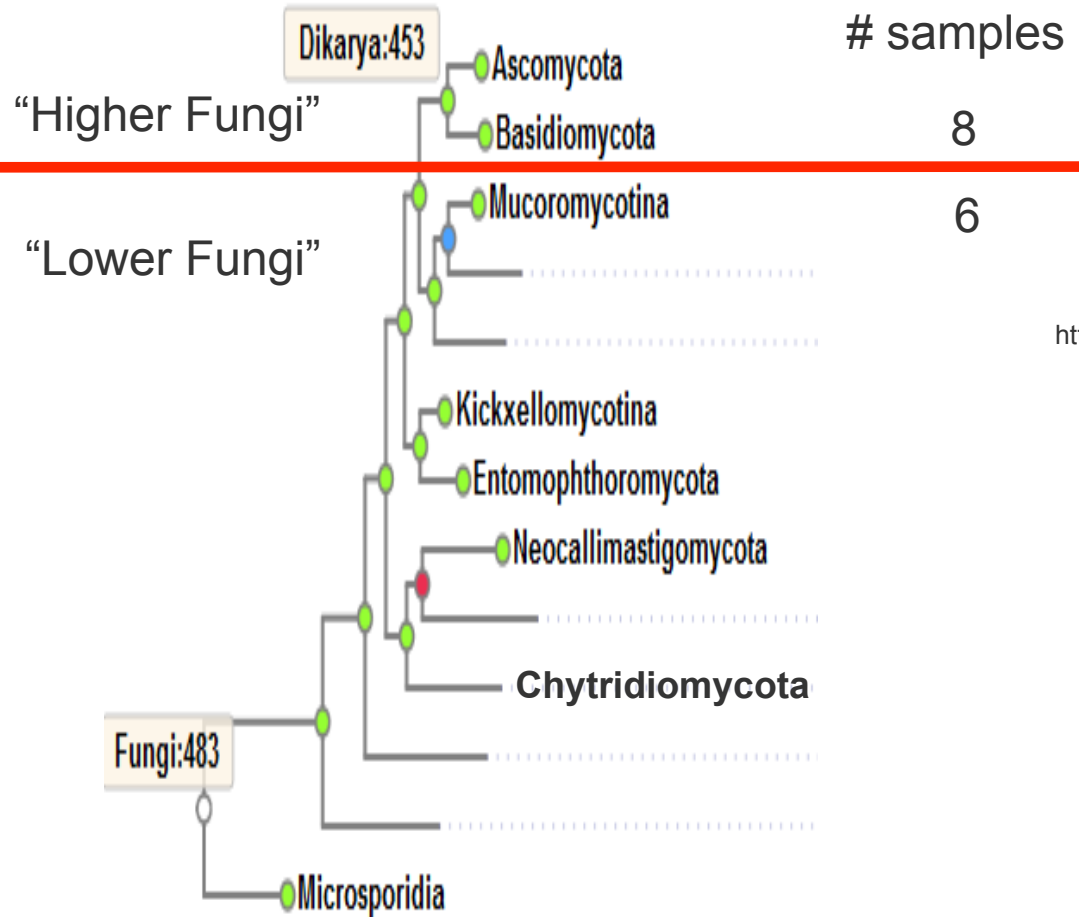
<https://en.wikipedia.org>

Methylation detection using PacBio

- **PacBio reads methylation status during sequencing**
 - Measures rate of DNA base incorporation (delayed by methylation)
 - Routine at JGI for bacterial genome sequencing (For example, Blow et al., 2016)



Adenine methylation discovered in lower fungi

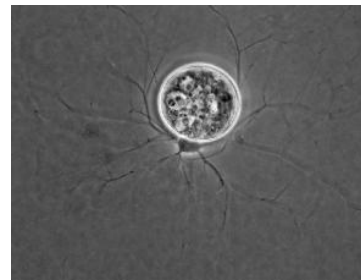


Higher fungi

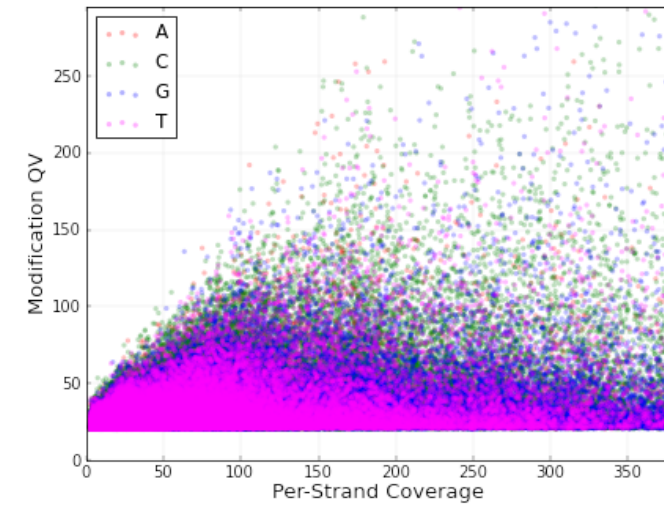


<http://genome.jgi.doe.gov/Amamu1>

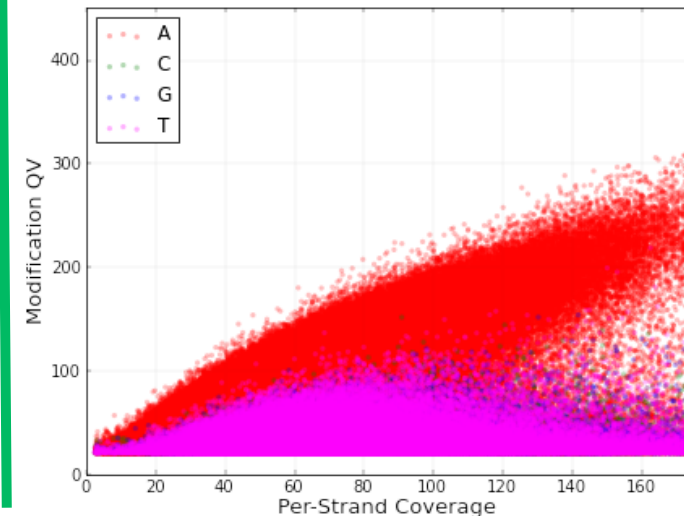
Lower fungi



<http://genome.jgi.doe.gov/Rhihy1>

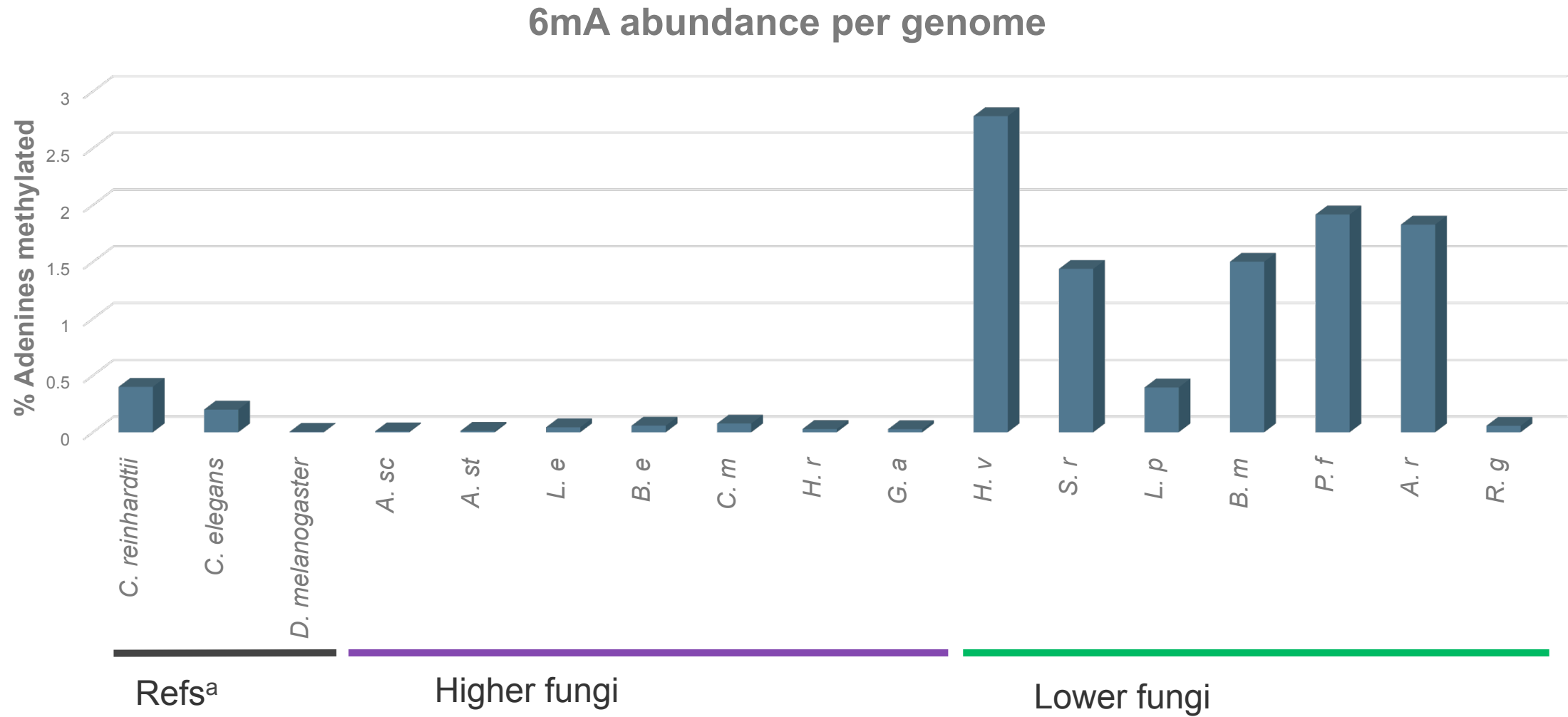


- Adenine
- Cytosine
- Guanine
- Thymine



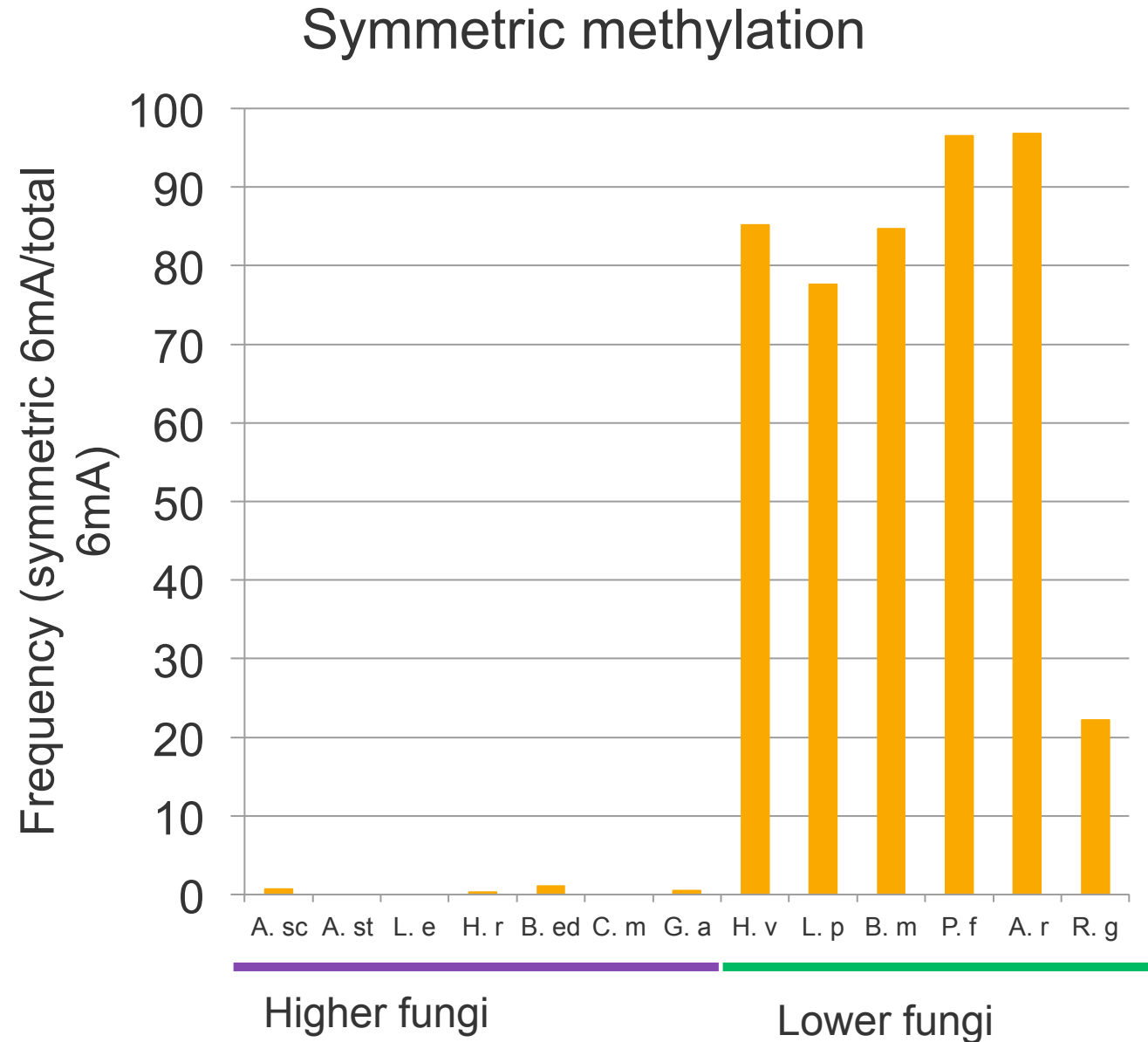
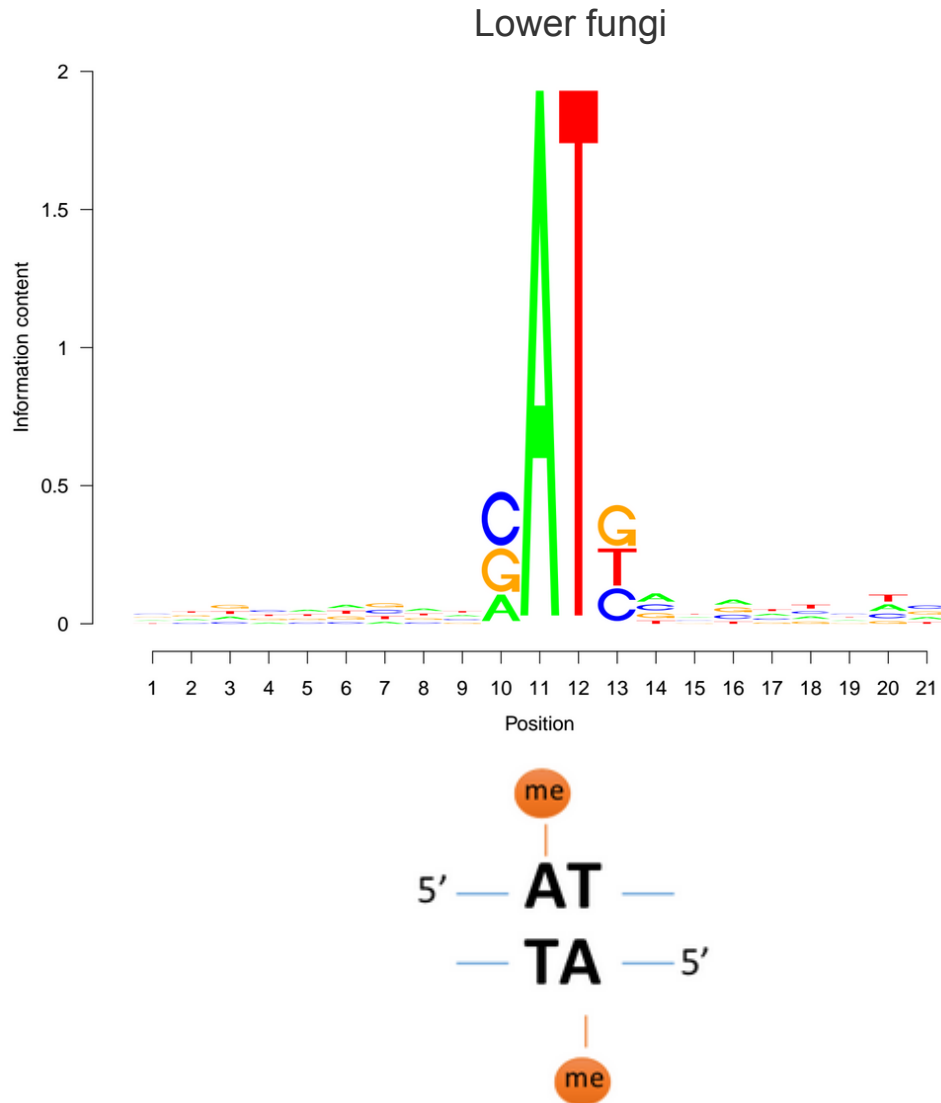
<http://genome.jgi.doe.gov/mycocosm/species-tree/tree?organism=fungi>

6mA distribution across fungi

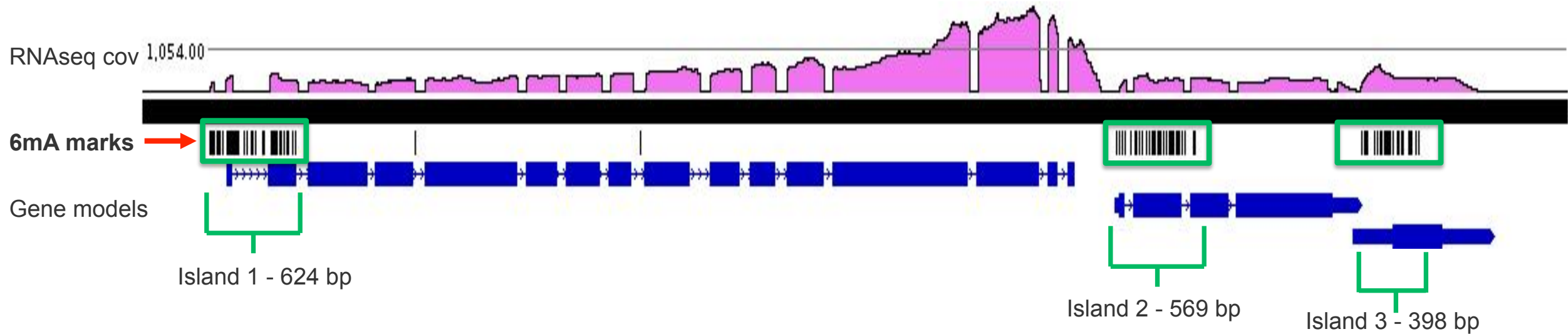


^a Fu et al., 2015 (Cell)
Zhang et al., 2015 (Cell)
Greer et al., 2015 (Cell)

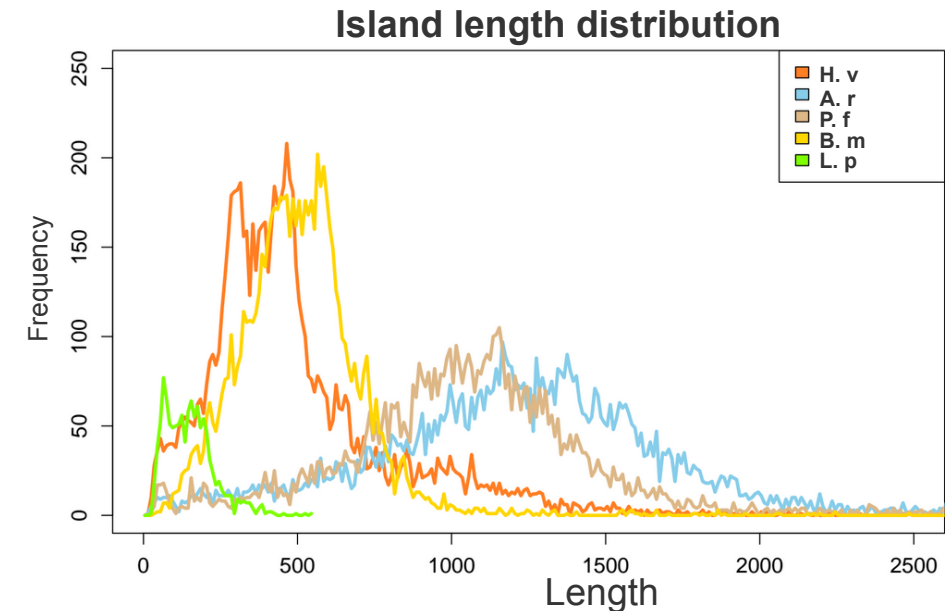
Conserved 6mA motif in lower fungi



Nearly all methylated sites are found in islands

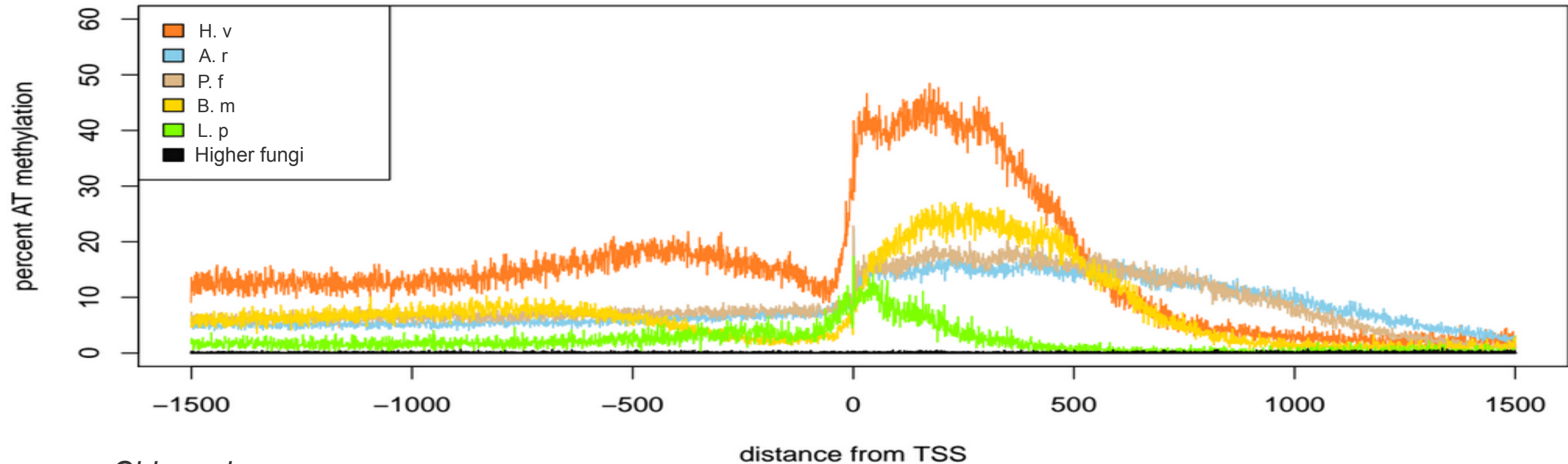


- >90% of methylation marks concentrate into 'islands' for all lower fungi
- Islands located mostly at protein-coding gene promoters
- Found at >50% of genes in most genomes

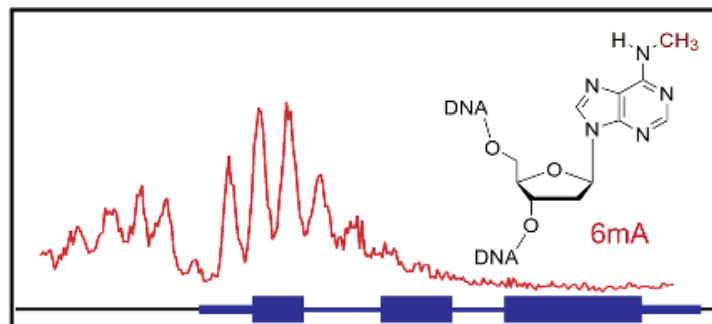


TSS-biased methylation

- Methylation marks concentrate at (and downstream of) Transcriptional start site

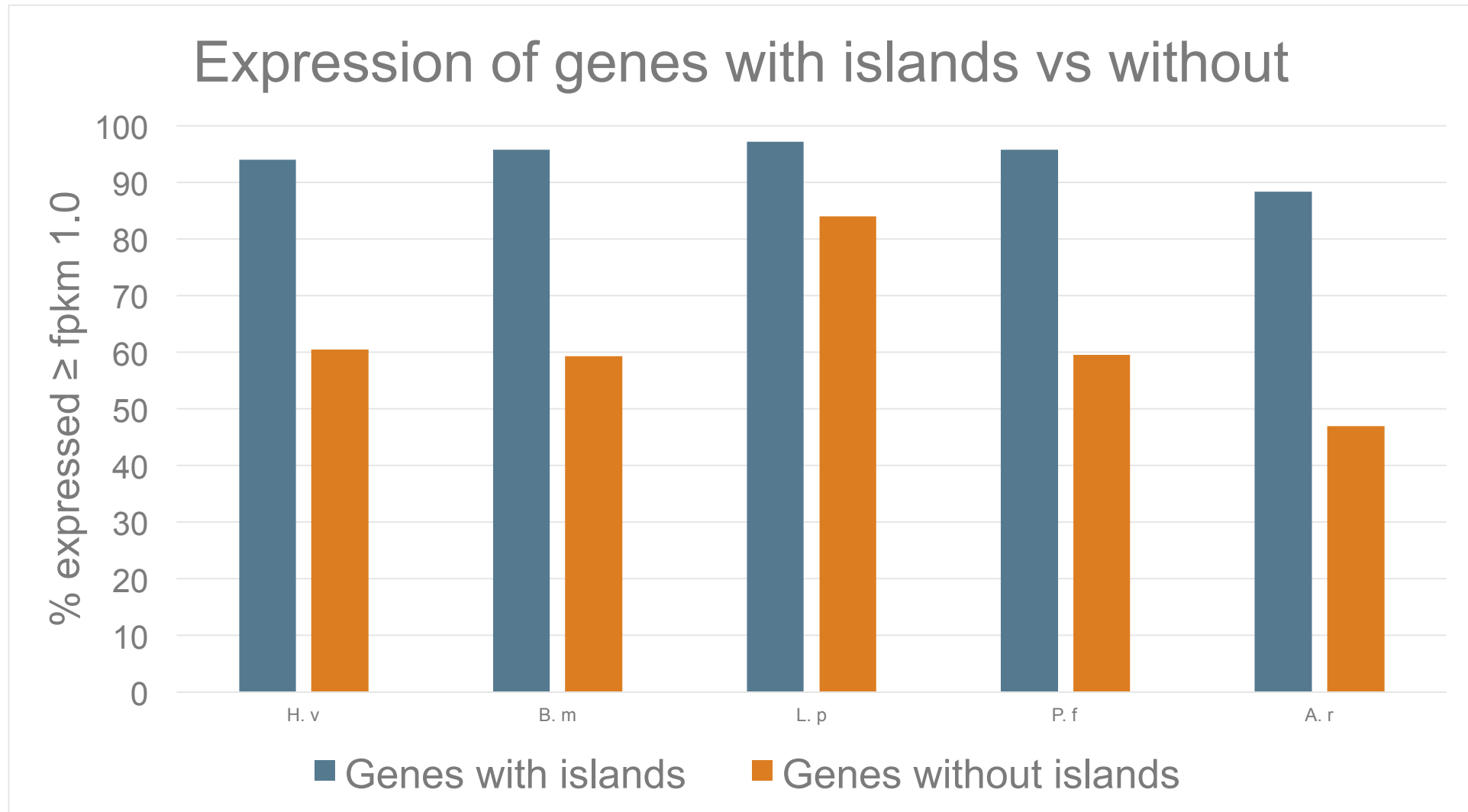


Chlamydomonas

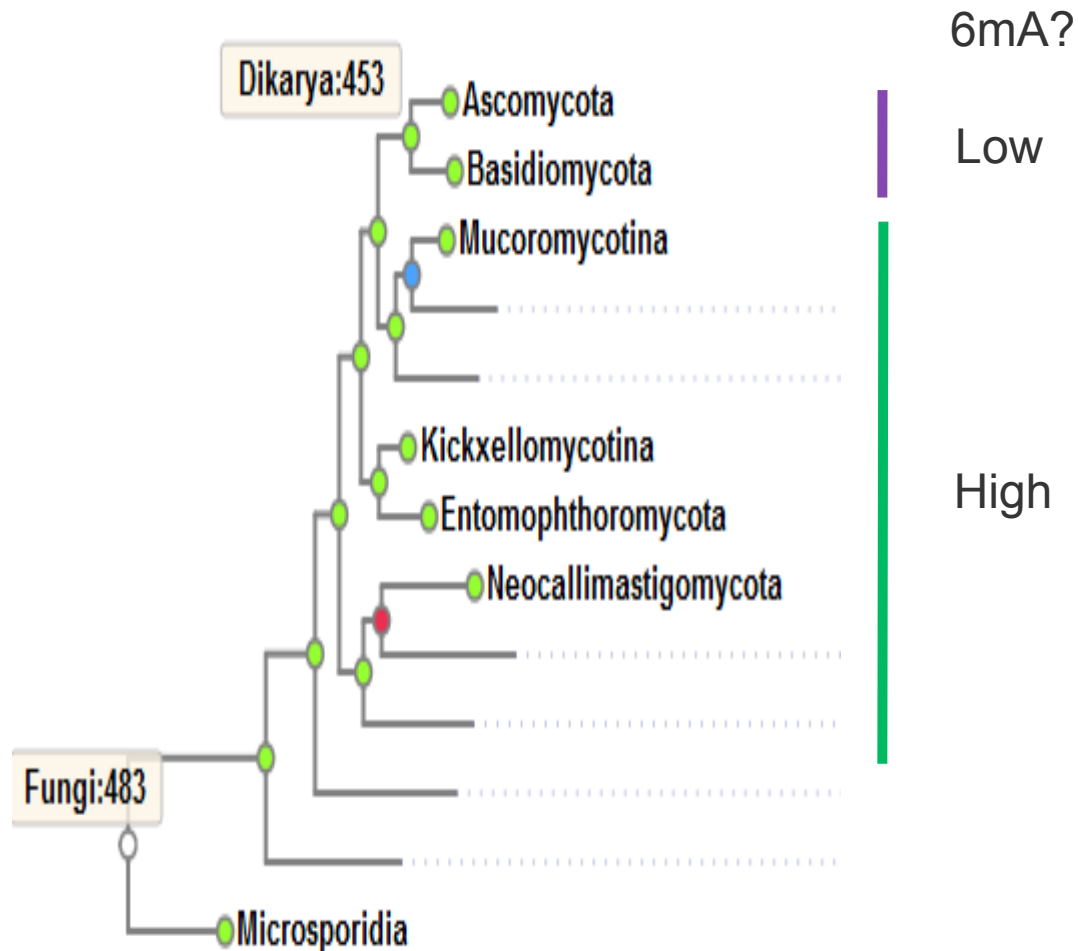


- Expression coincident with island starts
- Distinct from profile observed in *Chlamydomonas*

Genes with 6mA islands are almost always expressed



Summary



- **PacBio is a powerful tool for discovery of 6mA**
 - R-package under development for downstream analysis
- **Available through the CSP program**

Using this tool, in fungi we found:

- **6mA is symmetric and frequent in lower fungi**
 - Up to 7x higher than in other eukaryotes so far
- **6mA concentrates in 'islands' found at the transcriptional start site of coding genes**
- **Qualitative impact on gene expression**

Acknowledgements



JGI:

- Igor Grigoriev
- Govindarajan Ramamoorthy
- Asaf Salamov
- William Sullivan
- JGI Fungal Annotation Team

External collaborators:

- Tim James
- Jason Stajich
- Andrii Gryganskyi
- Joey Spatafora
- Michelle O'Malley

Funding:

- DOE - JGI

