Table S1. Descriptive statistics of 6mA distribution in the genome, Related to Figure 1.
Properties of 6mA distribution in nucleosome linkers. In *Oxytricha*, methyl cluster 1 = between 5' chromosome end and +1 nucleosome; methyl cluster 2 = between +1 and +2 nucleosome; methyl cluster 3 = between +2 and +3 nucleosome. In *Tetrahymena*, methyl cluster 1 = between +1 and +2 nucleosome; methyl cluster 2 = between +2 and +3 nucleosome; methyl cluster 3 = between +3 and +4 nucleosome. Consensus +1/+2/+3/+4 nucleosome positions: 193, 402, 618, 837 bp downstream of *Oxytricha* 5' chromosome ends; 112, 304, 497, 698 bp downstream of *Tetrahymena* TSSs.

	Number of 6mA sites									
	Oxytricha					***Tetrahymena***				
	Minimum	**Maximum**	**Median**	**Mean**	**Standard Deviation**	**Minimum**	**Maximum**	**Median**	**Mean**	**Standard Deviation**
Methyl Cluster 1	0	14	2	2.03	2.27	0	27	10	9.68	6.10
Methyl Cluster 2	0	24	6	5.99	4.24	0	26	9	8.78	5.78
Methyl Cluster 3	0	16	2	2.49	2.91	0	25	5	5.75	5.53

Table S2. Candidate genes in ciliates, Related to Figures 2 and 6.

The Uniprot ID of each gene is listed. The *Oxytricha* macronuclear genome encodes five genes belonging to the MT-A70 family (Iyer et al., 2016; Swart et al., 2013). Such genes commonly function as RNA m^6A MTases in eukaryotes, having evolved from m.MunI-like MTases in bacterial restriction-modification systems (Iyer et al., 2016). An MT-A70 gene belonging to the METTL4 subclade, DAMT-1, is a putative 6mA methyltransferase in *C. elegans* (Greer et al., 2015). However, none of the *Oxytricha* MT-A70 genes in this Table cluster together with METTL4 on a phylogenetic tree (Figures 2A and S2G). The *Oxytricha* genome also contains homologs of a structurally distinct RNA m^6A MTase, METTL16, which was reported to methylate U6 snRNA (Table S2) (Pendleton et al., 2017; Warda et al., 2017). Another candidate, N6AMT1 – which does not contain an MT-A70 domain – was recently found to mediate DNA 6mA methylation in human cells (Xiao et al., 2018). An N6AMT1 homolog is also present in the *Oxytricha* genome. Accessory factors refer to the p1 and p2 proteins, which are necessary for 6mA methylation by MTA1 and MTA9 *in vitro*. The UniProt IDs of putative ISWI homologs in *Oxytricha* and *Tetrahymena* are also listed.

MT-A70 genes in *Oxytricha trifallax*

UniProt ID	Gene name in this study	OxyDB gene name
J9IF92_9SPIT	MTA1	Contig12701.0.0.g16
J9IGS7_9SPIT	TAMT-1	Contig17486.0.g100
J9J9V7_9SPIT	MTA1-B	Contig16314.0.g25
J9HW68_9SPIT	MTA9	Contig1237.1.g126
J9IMU5_9SPIT	MTA9-B	Contig17419.0.g36

MT-A70 genes in *Tetrahymena thermophila*

UniProt ID	Gene name in this study	*Tetrahymena* Genome Database gene name
Q22GC0_TETTS	MTA1	TTHERM_00704040
Q23TW8_TETTS	MTA2	TTHERM_00962190
I7LVP8_TETTS	MTA3 / TAMT-1-B	TTHERM_00136470
I7MGX6_TETTS	MTA4	TTHERM_00558100
Q23RE0_TETTS	MTA5 / TAMT-1	TTHERM_00388490
I7MIF9_TETTS	MTA9	TTHERM_00301770
Q22XT1_TETTS	MTA9-B	TTHERM_01005150

METTL16 homologs in *Oxytricha trifallax*

UniProt ID	OxyDB gene name
J9F3J7_9SPIT	Contig11945.0.g48
J9J5P9_9SPIT	Contig7462.0.g41
J9III0_9SPIT	Contig4244.0.g39

N6AMT1 homologs in *Oxytricha trifallax*

UniProt ID	OyDB gene name
J9IFV1_9SPIT	Contig7751.0.g12

Accessory factor genes in *Tetrahymena thermophila*

UniProt ID	Gene name in this study	*Tetrahymena* Genome Database gene name
Q22VV9_TETTS	p1	TTHERM_00161750
I7M8B9_TETTS	p2	TTHERM_00439330

ISWI homologs in *Oxytricha trifallax* and *Tetrahymena thermophila*

UniProt ID	OxyDB gene name	*Tetrahymena* Genome Database gene name
I7M280_TETTS		TTHERM_00137610
J9FBJ2_9SPIT	Contig11737.0.g12	

Oxytricha genome database (OxyDB): http://oxy.ciliate.org/index.php/home/welcome
Tetrahymena genome database: http://ciliate.org/index.php/home/welcome

Table S3. Mass spectrometry analysis of MTA1, MTA9, p1, and p2 proteins, related to Figure 2.
Percentage of each polypeptide that is covered by peptide data is calculated. “Low Salt Sample” and “High Salt Sample” correspond to partially purified nuclear extracts that elute as two distinct peaks of activity from a Q sepharose anion exchange column (Figure 2C).

Data from Low Salt Fraction

UniProt ID	Gene name in this study	% of protein covered by peptide data from LC-MS/MS experiment
Q22GC0_TETTS	MTA1	78.8%
I7MIF9_TETTS	MTA9	46.3%
Q22VV9_TETTS	p1	41.9%
I7M8B9_TETTS	p2	81.7%

Data from High Salt Fraction

UniProt ID	Gene name in this study	% of protein covered by peptide data from LC-MS/MS experiment
Q22GC0_TETTS	MTA1	69.9%
I7MIF9_TETTS	MTA9	72.2%
Q22VV9_TETTS	p1	55.3%
I7M8B9_TETTS	p2	93.4%

Table S4. Descriptive statistics of reference genomes, Related to Figure 4.
Properties of *Oxytricha* chromosomes in native genomic DNA and mini-genome DNA. "+/-" indicates one standard deviation above or below the mean.

	Native genomic DNA	Mini-genome DNA
Chromosome length (bp)	**2449 +/- 742** Min = 1155 Max = 6494	**2107 +/- 778** Min = 1201 Max = 4659
SMRT-seq coverage (x)	**177.4 +/- 117.0** Min = 75.1 Max = 1392.6	**205.3 +/- 136.1** Min = 77.8 Max = 918.4
Total number of 6mA marks in genome	46,322	2,344
6mA sites per chromosome	**12 +/- 8** Min = 0 Max = 73	**24 +/- 16** Min = 0 Max = 73
AT content (%)	**67.8 +/- 3.0** Min = 55.7 Max = 76.2	**66.5 +/- 2.7** Min = 60.2 Max = 72.2
RNAseq (FPKM)	**34.4 +/- 75.2** Min = 0.0 Max = 1444.5	**53.7 +/- 71.5** Min = 0.1 Max = 424.8