Supporting Information

Heyse et al. 10.1073/pnas.1009284107

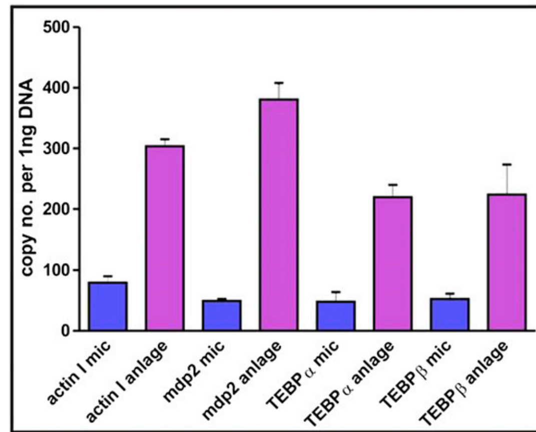

Fig. S1. Gene copy numbers in micronuclei and macronuclear anlagen of *Stylonychia.* DNA from micronuclei and macronuclear anlagen in the early polytene chromosome stage (Fig. 1) was isolated as described by Ammermann et al. (1), and the copy number of four genes (encoding actin I, mdp2, TEBPα, and TEBPβ) was determined by qRT-PCR. To avoid amplification of possible contaminating macronuclear DNA, micronucleus-specific primers were used (Table S1 and Fig. S2). In *Stylonychia,* only ~30% of micronuclear chromosomes enter the first DNA amplification stage; the kinetic complexity of macronuclear anlagen DNA in the polytene chromosome stage is substantially reduced compared with that in micronuclear DNA (1), explaining why copy number/ng DNA in the macronuclear anlagen is higher than in micronuclear DNA. Because the DNA isolated from anlagen DNA is always derived from nuclei with variable polyteny, no exact copy number/nucleus can be given. As expected, in micronuclei, the estimated copy number corresponds to approximately one to two gene copies per micronucleus. All MDS are found in both nuclei in about equimolar amounts; the situation in the macronucleus is not mirrored, demonstrating that selective amplification does not occur during the first DNA amplification stage. Blue indicates copy number/ng in micronuclear DNA; purple indicates copy number/ng in anlagen DNA.

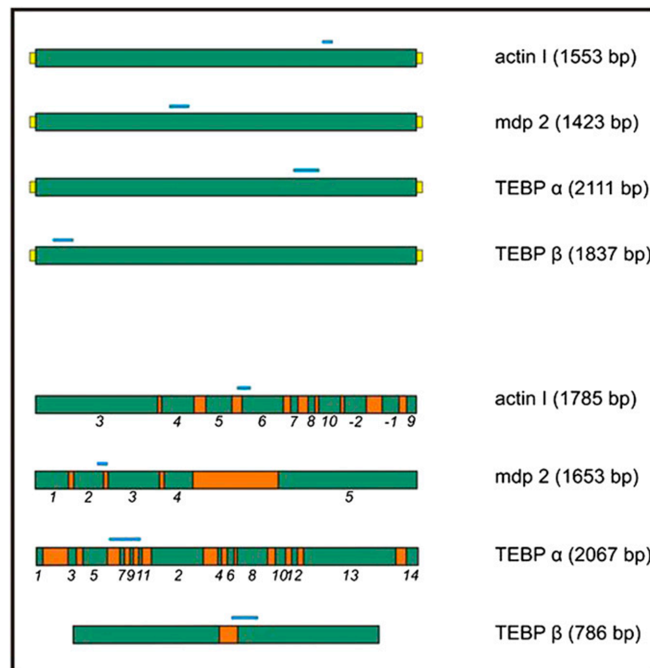

Fig. S2. Schematic illustration of macronuclear and micronuclear versions of four *Stylonychia* genes. MDSs are shown in green and are numbered according to their positions in the macronucleus. IESs are depicted in orange and telomeres in yellow. Blue bars indicate positions of qRT-PCR fragments. Primer pairs for amplification of micronuclear or anlagen DNA (*Lower*) were selected to amplify a micronucleus-specific fragment, and therefore always one primer is located in an IES. Total length of gene sequences in the macronucleus (*Upper*) or micronucleus (*Lower*) are given in brackets.

1. Ammermann D, Steinbrück G, von Berger L, Hennig W (1974) The development of the macronucleus in the ciliated protozoan Stylonychia mytilus. *Chromosoma* 45:401–429.

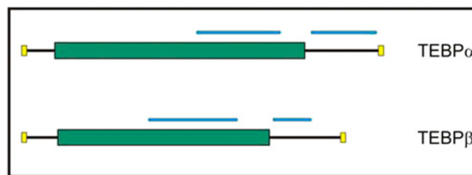

Fig. S3. Schematic illustration of DNA sequences used for RNAi experiments. Macronuclear versions of the *tebp*α and β nanochromosomes are depicted. Partial sequences of both genes were amplified and cloned into the vector L4440, which produces dsRNA of the inserted sequence. Bacteria expressing this dsRNA were fed to *Stylonychia* cells to induce RNAi. The coding region of each gene is shown in green; the untranslated regions are shown as black bars with attached telomeric sequences (yellow). Blue bars represent the part of the gene from which dsRNA was produced. For each gene, one sequence was taken from the coding region and one sequence from the 3′untranslated region.

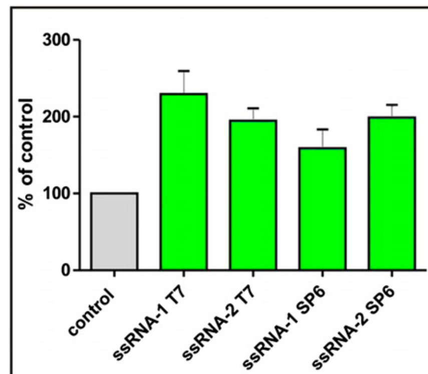

Fig. S4. Increase in copy numbers of the *actin I* nanochromosome after microinjection of ssRNA. Columns show the increase in copy numbers after injection of *actin I* template molecules. Here, ssRNA was injected into early exconjugant cells. Both orientations (transcribed with either T7 or SP6 RNA polymerase) were tested in different injection experiments; for each orientation, two clones are shown ("ssRNA-1 or -2"). "Control" cells were selected from a population of cells that were not injected but did conjugate. This control was set to 100%. n = 12. Error bars represent SD.

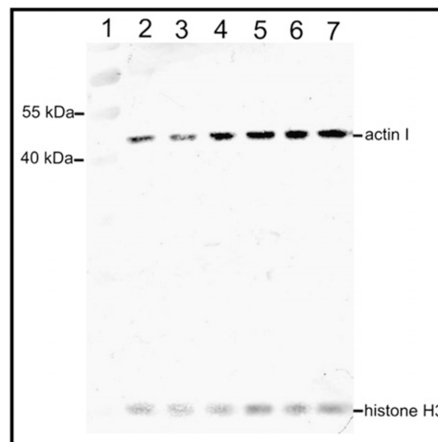

Fig. S5. Quantitative Western blot analysis of the actin I protein after template injection. After injection of *actin I* templates and several vegetative cell divisions, whole proteins of 200 cells per slot were separated by SDS/PAGE. Western blot analysis was then performed as previously described (1). Actin was stained by using a polyclonal anti–β-actin antibody (Abcam); as an internal control, histone H3 was stained using a polyclonal antihistone H3 antibody (Santa Cruz Biotechnologies). Lane 1 represents prestained protein marker (Fermentas); lane 2, control cells; lane 3, control cells injected with Pringsheim solution; lanes 4–7, cells after injection of DNA- (lane 4) and ssRNA-template (lanes 5–7). In lanes 5–7, cells of three independent injection experiments are shown.

1. Jönsson F, Postberg J, Schaffitzel C, Lipps HJ (2002) Organization of the macronuclear gene-sized pieces of stichotrichous ciliates into a higher order structure via telomere-matrix interactions. *Chromosome Res* 10:445–453.

Table S1. Primer sequences used in the experiments described

Purpose	Target/primer	Sequence 5′ to 3′
MAC qRT-PCR		
	Hsp70Mac-for-1	AAGTTTGGAGTGTTCGAAATTGA
	Hsp70Mac-rev-1	GACATGGCCGAGAGGTCTTA
	Hsp70Mac-for-2	AACGCCAGAGCTCTCAGAAG
	Hsp70Mac-rev-2	CTTGGCTCTTGAGATTTGGG
	TEBPβMac-for-1	CAACAATGAGCAAGGGTCAA
	TEBPβMac-rev-1	GCCTTGGAAACCTTGTTGAA
	TEBPβMac-for-2	TTCTTCAACCAAGGCGGTGA
	TEBPβMac-rev-2	CGATGTTGACGTTGCGGAAC
	TEBPαMac-for	GCTCTTAAACTCAAGTTCCCACAC
	TEBPαMac-rev	TTTGAAGCACTGACGAAGGTAACAA
	Mdp2Mac-for	GCAGGCTTTTGCAGAAAACA
	Mdp2Mac-rev	ATGCAGCATTCCTAGCCTCA
	PiwiMac-for	TCACTCATGAGAGGATTGAGATTCGA
	PiwiMac-rev	CTAGTGAGTGAGCTTTTGCAGAGTTG
	DNAPolαMac-for	GAAAATCGGCTAAAAATCAGGTG
	DNAPolαMac-rev	TCCACCCTCTCTAGCAGCT
	ActinIMac-for	TTGCTGGCGAAGGTTGAGAG
	ActinIMac-rev	TGCCAGCCCAGACAGAAGAT
	rDNAMac-for-1	CTGGTTGATCCTGCCAGTAG
	rDNAMac-rev-1	CCACGGTTATCCATGTAAATTC
	rDNAMac-for-2	CTCCTTTGTTGGTTTGAGGG
	rDNAMac-rev-2	AACATCCTTGGCAAATGCTT
	1.1kb gene Mac-for-1	AACAGCCTATTCCCCCTGAT
	1.1kb gene Mac-rev-1	GTGTCAACGCCGAAGAATTT
	1.1kb gene Mac-for-2	ACATCGCAATTGTGCCAGA
	1.1kb gene Mac-rev-2	CCAGATCGTCTTGTTTTGGTC
MIC+Anlagen qRT-PCR		
	ActinIMic-for	TTGATTGGAGTTTAATGATTGGTG
	ActinIMic-rev	TGGACTTTGGTGCTCTGTTTT
	Mdp2Mic-for	ACTCACAGCAAGGGGGATA
	Mdp2Mic-rev	CTGGCTAAAATTAAAAATATATCTCC
	TEBPβMic-for	GAATCTATATTGCATTGACTATA
	TEBPβMic-rev	TTTGAGATATCTGGAAGTGACTCTTA
	TEBPαMic-for	CCATTCGTTTGTCATTTTGTTACTTATAATTAAATTATATC
	TEBPαMic-rev	AAGTAGCTGATCTAATTCTAACAA
Cloning standards		
	Hsp70Mac-for-1	AAGTTTGGAGTGTTCGAAATTGA
	Hsp70clo-rev	CATACCGCCTGGCATTC
	TEBPβMac(/Mic)-for-1	CAACAATGAGCAAGGGTCAA
	TEBPβcloMac-rev	CTCCTTTGACCTTTGGTTGA
	TEBPβcloMic-rev	TTCTGCTTGATGGCTTGTTG
	TEBPαcloMac-for	TTATTGGTAGTCGTTAGATTCAAG
	TEBPαcloMic-for	CCATTCGTTTGTCATTTTGTTACTTATAATTAAATTATATC
	TEBPαclo-rev	GTAAGGAGTTCGTTGTATTCCTCGAG
	Mdp2clo-for	CTCACTAATGAATTTCGAGGTTGAG
	Mdp2clo-rev	ATCAGTCTCTGAGGGAAATAGGC
	Piwiclo-for	CCGTAGTTTCAGAATTCGACAGG
	Piwiclo-rev	TTTGGTGGCTAACCATTTAAGAAA
	DNAPolαclo-for	TCATCCAGCAGGGACCTTTA
	DNAPolαclo-rev	TTCTTTTTACCGCCAACCTC
	ActinIclo-for	CAATCGTTGGTAGACCCAAGAAC
	ActinIclo-rev	AGCAAGGATATTTAAGTAAGGGC
	rDNAMac-for-1	CTGGTTGATCCTGCCAGTAG
	rDNAclo-rev	GGTTCACCTACGGAAACCTTG
	1.1kb gene clo-for	GGCTACACTGGCCAGGTTC
	1.1kb gene clo-rev	CCTTTCAAAAATATATACTATATTTGAAATAC
Cloning RNAi coding		
	TEBPαMac-for	GCTCTTAAACTCAAGTTCCCACAC
	TEBPαRNAi-rev	CTAGGTGTAGAGGAGAATTCTGTA
	TEBPβRNAi-for	GCATCAAGCTTGATATCTCAAAGCTCTCTGG
	TEBPβRNAi-rev	GGTAACTCGAGCCATTGTCATTTGATCAGTG

Table S1. Cont.

Purpose	Target/primer	Sequence 5′ to 3′
Cloning RNAi 3′UTR		
	TEBPα3′RNAi-for	GCGAAGCTTCGATTTTGAAAACCATAAAAG
	TEBPα3′RNAi-rev	GAACTCGAGACCAGATTTAACTCGAAGATG
	TEBPβ3′RNAi-for	CCTAATTCCTCGTGTGATAAATGC
	TEBPβ3′RNAi-rev	CATTATACCTCATTGAACGAGCTT
Cloning injections		
	Actin I P5′ tel	AAAACCCCTATAGAGAGTATTAGATGTATTGATTAGG
	Actin I P3′ tel	AAAACCCCATTTGATGGAATTTAGTATAAATAAGTGG

Table S2. Nucleotide sequences clones into vector L4440 and used in RNAi experiments

Source of fragment for RNAi	Primers	Sequence 5′ to 3′
tebpα coding region	TEBPα Mac-for x TEBPα RNAi-rev	GCTCTTAAACTCAAGTTCCCACACTTGAGAGCTGGAGA AGTTGTTAGAATTAGATCAGCTACTTATGATGAGACCT CAACCCAAAAGAAGGTCCTCCTTCTAAGTCACTACTCC AACATTGTTACCTTCGTAAGTGCTTCAAAGCTTGCCAA GGAAATCAAGGGTAAAGTCACTGATGATAAATCAGTTG AGAAGGCTGCACTCAAGCAAGATGTTAGCTTGAGCGT TGTCGTCCTCACTGAAGTCGACAAGAAGCATGCTGGT CTCCCAACCCACTCACTTCAAGATTTATTCCACAACGC TGATACTGATAAGGAAATCTCAAGCAAAGACACCTTCA GAACTCAATTCTACATTACCAGAGTTGAACCAGTTGAT GTTAAGGAATGGGTTAAGTCATATGACAGAAAATCAAA GAAGGCCTCATCCCACAAGGGTGCTGCAGCCAAGAGT GGAGAGAACATTTTCCAAGTTCAATTCCTCGTCAAGGA TGCCTCAACTCAACTTAACAACAACACCTACAGAATTC TCCTCTACACCTAG
tebpα 3′UTR	TEBPα 3′RNAi-for x TEBPα 3′RNAi-rev	GCGAAGCTTCGATTTTGAAAACCATAAAAGGTTAAGTA ATGAAACCTAATGATTATTATATCTTCTTATAAAAGTTAA AAATACTCCTAAGCTCTTTAAATTCAATTTTTAAAACTTA AAATTCAAAAAGTAATTAATAATCCTTGTTAGGAATTTA ATTTGATTCATGAACCTGTTATATATTATGATTAGTAAC TTTAGAAGTTAATAAGTTTTCTAGCAGCCTAATCATTCT ATATTCATGCTTTTAATAACCTAAATTGGGGAAATTTAT TGTCTATTTCAGGAGACTATTCTTGTATATTTGTAAATA TAAGTTGAAATTTGTATAAATAAATGAGAAATAGCCTTA GTAATTATCAATACCCATCTTCGAGTTAAATCTGGTCTC GAGTTC
tebpβ coding region	TEBPβ RNAi-for x TEBPβ RNAi-rev	GCATCAAGCTTGATATCTCAAAGCTCTCTGGTGCTGAT GCTGCTGGCAAGAAGACCAAGGTCGATGGAGGTATTG TTAAAACCGGTGCTTCCAAGGGTGACGAGTTCGCAGA CTTCTCATTCAAGGAGGGCAGCACTGCCGTTCTCAAG ATCCAAGACATCTTTGTCCAAGAGAAAGGCAAAGATGC TCTTAAGAGAATTCAAGATGCTCAAGTCGACAGCGTTC AAGCCCAACCAAAGGTTAAGGGAGGTGCTAAAGGCAA GAAGAAGGCCGCCACCAAGTCAGCCACCAAGAAGACT GTTGCTGCCAAGAAAACTGCCGAGTCTGCTGACGTTA GAAAGAGCGTTGACAAGATCGTTAAATATACTCCTAAC AAGCCATCAAGCAGAAAGGAGACCCCATAAAAGAGCC AATCTGCTCCAGCTGCTGGCAAGTCATCAGCCAAGAG AACCACCACCGGCTCAAAGACCAAGATCCCAGCCAAC CCATCCCCAAGCGGAAAGAAGTCAACCAAGACCACTG ATCAAATGACAATGGCTCGAGTTACCA
tebpβ 3′UTR	TEBPβ 3′RNAi-for x TEBPβ 3′RNAi-rev	TCCTAATTCCTCGTGTGATAAATGCTGATGTTATTATAC TAATTCTCTTAAATGAGTATTTTCATAACACTTAAAAAAA CTCAGTTTCTTTAACTCACAAAATATTCATGATGATTTT TGAATGGCTGGCTATTCTGGTATTCTAGAATAGGCTAT TCAGGATGACACTAACTTTAAATTTTAAGTCTCAATCAC ATTCAATTAAAAGCTCGTTCAATGAGGTATAATG

Table S3. GenBank accession numbers for analyzed genes

	GenBank accession no.	GenBank accession no.
Gene	Macronucleus	Micronucleus
hsp70	AF227962	
TEBPβ	AF190703	HM437167
TEBPα	AY751782	HM920148
mdp2	AY261997	GU111958
Piwi (mdp1)	AY261996	
DNA polymerase α	AF194338	
actin I	DQ108617	DQ108616
rDNA	AF164124	
1.1kb gene	X72955	