SUPPLEMENTARY INFORMATION TO:

Multimerization properties of PiggyMac, a domesticated piggyBac transposase involved in programmed genome rearrangements

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SUPPLEMENTARY MATERIAL AND METHODS

Construction of GFP fusion transgenes

$PGM\text{-}GFP$ was constructed by replacing the $Afl\text{III}\text{-}Xho\text{I}$ fragment of $pBL49g$ (1) by an $Afl\text{III}\text{-}Xho\text{I}$ PCR fragment carrying the 3’ end of $PGM$ fused (in frame) to the $eGFP$ sequence adapted to $Paramecium$ codon usage (2), followed by $PGM$ transcription termination signals (Figure S1). $PGM^{\ast}\text{-}GFP$ is a derivative of $PGM\text{-}GFP$, in which the $Avr\text{II}\text{-}Bst\text{XI}$ fragment was replaced by a synthetic fragment (DNA2.0) containing a large number of silent substitutions (Figure S2), but designed to encode the same peptide. $PGM^{\ast\Delta A}\text{-}GFP$ was obtained by site-directed mutagenesis of $PGM^{\ast}\text{-}GFP$ using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene), with primers OMB422 to OMB427 (Table S1). $PGM^{\ast\Delta CCR}\text{-}GFP$ was cloned by replacing the $Bsm\text{I}\text{-}Cla\text{I}$ fragment of $PGM^{\ast}\text{-}GFP$ with a fragment amplified by trans-PCR using primers OMB433 and OMB561 to OMB563 (Table S1). $PGM^{\ast\Delta ACR}\text{-}GFP$ was constructed using the same procedure, with primers OMB433, and OMB563 to OMB565 (Table S1).

Copy number quantification of the $PGM\text{-}GFP$ transgene in microinjected cells.

Genomic DNA from a non-injected 51 $nd7\text{-}1$ clone was used as a reference to normalize all quantifications.

Because all genomic loci are amplified to similar levels in the $Paramecium$ somatic nucleus (3), we consider that the copy number of endogenous $PGM$ is equal to that of any other genomic region, such as gene $GSPATG00009109001$ that we used as a reference control (4). Following injection, the linearized transgene is trimmed and forms linear concatemers $in\ vivo$, in a head-to-tail or tail-to-tail configuration (5). Therefore, the hybridization pattern of a $PGM$ probe on Southern blots consists in multiple bands and a smeary background corresponding to the transgene, overlapping the single discrete band of endogenous $PGM$ (see below). For each sample, the total $PGM$ signal was quantified along the entire lane: it represents the sum of endogenous $PGM$ and copies of the $PGM\text{-}GFP$ transgene.
Figure Legend: Quantification of injected transgene copy numbers.

*Hind*III-digested total genomic DNA from microinjected cells was run in a 1% agarose gel. Following alkaline transfer to a Hybond N+ nylon membrane, the blot was hybridized with a *PGM* probe (Figure 1) and endogenous and total *PGM* signals were quantified as indicated in the text. Lanes labeled with cphg = 0 correspond to non-injected cells (ni).

For all microinjections performed into 51 *nd7-1*, transgene copy-numbers per haploid genome (cphg) were calculated using the following formula:

\[
cphg_{inj} = \frac{\left[ \frac{\text{PGM} \text{tot}}{\text{control}} \right]_i}{\left[ \frac{\text{PGM} \text{tot}}{\text{control}} \right]_{ni}} - 1
\]

where we subtracted 1 cphg corresponding to the endogenous *PGM* copy.

For microinjections into the Δ*PGM* strain, we used the same formula (where ni designates the values obtained for a non-deleted 51 nd7-1 reference clone), except that we did not subtract the endogenous *PGM* copy.

Supplemental Bibliography


LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1: Sequence of the AflII-XhoI insert carrying the eGFP-coding sequence inserted between the last 3’ codon and the TGA stop codon of the PGM gene
AflII and XhoI restriction sites are highlighted in grey, the eGFP-coding sequence is in green. Small caps: genomic sequence carrying PGM transcription termination signals.

Figure S2: Alignment of the synthetic AvrII-BstXI fragment from PGM* with the Paramecium genomic sequence
The synthetic fragment was designed in order to maximize the number of silent point mutations within the HindIII-NcoI fragment (corresponding to the dsRNA produced by RNAi-inducing bacteria), relative to the endogenous sequence. Note that the NcoI site (CCATGG) is embedded within the BstXI restriction site.
Red bars: nucleotide sequence identity between the two sequences within the AvrII-HindIII fragment. Blue bars: identical nucleotides within the HindIII-BstXI fragment. Blue dots: nucleotide substitutions between the two sequences.

Figure S3: Localization of the Pgm-GFP fusion encoded by the PGM*-GFP transgene during an autogamy time-course of injected WT cells
Localization of the Pgm-GFP fusion expressed from the PGM*-GFP transgene during autogamy in a control RNAi (ICL7). Microinjected cells express Pgm-GFP under endogenous PGM transcription signals. Cells were fixed at different time points during an autogamy time-course (Figure S4A), in order to get developing MACs at different stages. To compare the intensities of GFP fluorescence, signals were acquired with the same exposure time, and identical window settings were applied to the image display using both Photoshop and ImageJ softwares. All panels are on the same scale (scale bar = 5 µm). When applicable, yellow arrowheads point to the developing MACs that are enlarged in the bottom right inserts. Other developing MACs are marked with white arrowheads.

Figure S4: Progression of autogamy stages in the different time-course experiments reported in this study
(A) Autogamy of wild-type cells upon ICL7 or PGM RNAi. Non-injected cells and cells harboring the PGM*-GFP transgene (cphg = 0.44) were grown in parallel in each indicated RNAi medium. For each time-point (St: starved cells; 0: 50% cells with a fragmented old
MAC; other time-points are in hours), the percentage of cells at each autogamy stage was determined following DAPI staining of fixed cells and fluorescence microscopy.

(B) Autogamy of ΔPGM cells expressing Pgm-GFP or its mutant derivatives. Non-injected cells, or cells harboring PG*M*-GFP (cphg = 22), PG*M*-A3G-GFP (cphg = 68), PG*M*-ΔCR-GFP (cphg = 29) or PG*M*-ΔCC-GFP (cphg = 21) were grown in standard culture medium and starved to trigger autogamy. Autogamy stages were monitored as described in (A).

Figure S5: Localization of the Pgm-GFP fusion in the developing MAC is consistent with the pattern of endogenous Pgm

(A) Localization of Pgm-GFP in live cells grown in standard culture medium. Before immobilization on microscope slides, autogamous cells were mixed with 2% methylcellulose and 5-µm calibrated beads (Fluka) to avoid crushing. Scale bar = 5 µm.

(B) Specific detection of endogenous Pgm using immunofluorescence microscopy. Non-injected cells were fixed between T5 and T10 during an autogamy time-course in control RNAi medium (ICL7: top panel; Figure S4A). T5 and T10 time-points correspond to the maximal level of Pgm in the population (Figure S6). The localization of endogenous Pgm is similar to the Pgm-GFP fusion protein (see panel A). The specificity of α-Pgm 2659 antibodies was confirmed by the absence of a fluorescent signal upon PGM RNAi (bottom panels). Scale bar = 5 µm.

(C) Dynamic pattern of Pgm localization during MAC development. For each individual developing MAC, confocal imaging was performed on the same slides as in panel B, as described in Materials and Methods. Due to asynchrony of autogamy in the population (Figure S4A), a wide range of developmental stages were observed. To reconstitute the progression of MAC development, new MACs were grouped into three classes according to their size (I: 20-30 µm²; II: 30-45 µm²; III: 45-60 µm²). At early and intermediate stages (I and II), the nucleoplasm showed a granular pattern with variably-sized foci, and conspicuous larger foci were associated with large DAPI-poor regions. At later stages (III), the immunofluorescence signal became weaker and more dispersed (panel d). The left panels show maximum intensity Z-projections of 36 serial optical sections of each new MAC. The right two panels on each row show the same optical section acquired at the maximal surface of the new MAC (α-Pgm, DAPI). Scale bar = 2 µm.

In panels A and B, yellow arrowheads point to the developing MACs that are enlarged in bottom right inserts. In each cell, the second new MAC is marked with a white arrowhead.
Figure S6: Expression pattern of endogenous Pgm during autogamy of wild-type cells
Top panel shows a western blot of total proteins extracted during an autogamy time-course of wild-type cells grown in a control RNAi (ICL7, see Figure S4A), revealed using purified α-Pgm 2659(AF) rabbit antibodies. In the bottom panel, the αTubulin signal was obtained for the same membrane using α-alpha Tub antibodies. Expected size for Pgm: 129 kD.

Figure S7: Induction of PGM somatic deletions in conjugating cells upon PGM RNAi
(A) Experimental strategy. For each mating-type, the RNAi-resistant PGM*-GFP transgene (hatched box) was microinjected into the MAC (black) of young cells (2 to 3 divisions after the previous autogamy) (step 1). Conjugation was induced between reactive mt7 and mt8 injected cells (step 2) and mating pairs were transferred to RNAi medium targeting endogenous PGM (step 3). Because mating pairs do not ingest bacteria, MIC meiosis and gametic nuclei exchange take place normally to give rise to the zygotic nucleus. In contrast, new MACs start to differentiate once exconjugants have separated and start to feed on RNAi-inducing bacteria. Thanks to expression of the complementing transgene from the old MAC, functional Pgm-GFP is produced throughout autogamy, allowing the recovery of viable exconjugants, in which new MICs (blue) and a new MAC (red) have developed from mitotic products of the zygotic nucleus. RNAi against endogenous PGM induces different levels of PGM deletion in the new MACs of post-mating exconjugants.

(B) Screening for ΔPGM cells. Eleven independent exconjugants obtained as described in (A) were grown for 20 to 25 divisions in standard culture medium and starved to induce autogamy. For each exconjugant, the percentage of post-autogamous progeny harboring a functional new MAC is represented in the histogram. Exconjugants carrying a complete PGM somatic deletion are not expected to yield viable sexual progeny after autogamy, a phenotype exhibited by clones #5 and 6. Wild-type non-deleted cells (WT) were used as a control.

(C) PCR analysis of the PGM locus in clone #6. Deletion of the majority of PGM copies in the MAC can be detected by PCR amplification using primers OMB635 and OMB637, which hybridize in the flanking DNA, ~1-kb from PGM start and stop codons, respectively. Genomic DNA extracted from a WT clone was used as a non-deleted control (expected PCR product size for the non-deleted locus: 5.18 kb). The lower bands corresponding to heterogeneous PGM deletion products are defined by a brace. Size ladder: 1kb Plus DNA ladder (Invitrogen).

(D) Map of the genomic PGM locus. PGM and its two flanking genes are annotated below the diagram. Dark arrows (not to scale) indicate the position of the PCR primers used in (C).
Figure S8: Pgm$_{3A}$-GFP and Pgm$_{ΔCR}$-GFP proteins expressed in ΔPGM cells accumulate at late time points during autogamy

(A) Western blot of whole proteins extracted at different time points during an autogamy time-course from ΔPGM cells injected with WT, ΔCC or ΔCR transgenes (see Figure S4B). Fusion proteins were detected using α-GFP antibodies. Bottom panel: same western blot, following stripping and incubation with α-alpha Tub antibodies.

(B) Aberrant accumulation of Pgm$_{3A}$-GFP and Pgm$_{ΔCR}$-GFP at late autogamy stages. For each microinjected clone, total proteins were extracted at different time-points during autogamy (see Figure S4B) and loaded on 0.1% SDS 8% polyacrylamide gels. Western blots were first incubated with α-Tub monoclonal antibody (bottom panel) and stripped before detection of Pgm variants using α-Pgm 2659 antibodies. Black arrowheads indicate the position of each GFP fusion.

Figure S9: Pgm$_{ΔCC}$ partially restores IES excision upon dilution of PGM RNAi-inducing bacteria

(A) Detection of residual Pgm during autogamy of WT cells fed on indicated dilutions of PGM RNAi-inducing bacteria in control RNAi medium targeting the ND7 gene. Total protein extracts were prepared in boiling 10% SDS from a population of autogamous cells at T5 (i.e. 5 hrs after 50% of cells reached old MAC fragmentation stage). The immunoblot was incubated with α-Pgm 2659-GP antibodies for Pgm detection (top) and stripped before incubation with α-alpha Tub monoclonal antibody (bottom).

(B) Percentage of post-autogamous progeny with a functional new MAC for non-injected cells (cphg = 0) or cells harboring different copy-numbers of the ΔCC transgene. Each individual clone was submitted to control RNAi (0), or full (1x) or partial PGM RNAi (0,35x dilution of PGM RNAi-inducing bacteria). Survival of sexual progeny following autogamy was tested as indicated in the Materials and Methods section.

(C) Pgm$_{ΔCC}$ does not fully restore excision of IES 51A6649 in complete or partial PGM knock-downs. The ΔCC transgene was micro-injected into the somatic MAC of individual vegetative cells from a clonal population. Transformed clones harboring different transgene copy-numbers (same as in B) were submitted to control RNAi (0), or full (1x) or partial PGM RNAi (0,35x). Retention of IES 51A6649 in the new MAC is detected under both PGM RNAi conditions, following PCR amplification from total genomic DNA extracted from late
autogamous cells (at ~T72), using PCR primers OMB101 and OMB102 (Table S1). PCR conditions were as indicated in the Materials and Methods section.
Figure S1. Sequence of the *AflII*-XhoI insert carrying the eGFP-coding sequence inserted between the last 3’ codon and the TGA stop codon of the *PGM* gene.

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A

Sexual cycle (conjugation)

PGM*

RNAi against endogenous PGM

1. WT progeny with a functional new Mac (%)

B

C

WT ΔPGM

5.18 kb

D

2 kbp

287k 288k 289k 290k 291k 292k

GSPATG00016626001 PiggyMac GSPTAG00016628001

Dubois et al. Figure S7

Sexual cycle (conjugation)
A

WT | ΔCC | ΔCR

| kDa | 20 | 70 | 20 | 70 | hrs |

| 250 | 150 | 100 |

WT-GFP
ΔCR-GFP
ΔCC-GFP

α-GFP
α-alpha Tub

B

WT | 3A | ΔCR | ΔCC

| kDa | 20 | 70 | 0 | 20 | 70 (hrs) | 0 | 20 | 70 |

| 250 | 150 | 100 |

α-Pgm
α-Tub
A) Dilution of PGM RNAi medium

B) % progeny with functional new MAC

C) Comparison of genomic DNA fragments (bp) with control and PGM RNAi treatment.
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Table S1: Oligonucleotides used in the study