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## Interaction of the Cysteine-Rich Domain of the Paramecium domesticated transposase PiggyMac with histone H3

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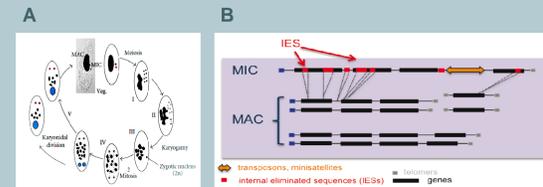
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## Background

Transposases catalyze the DNA strand breakage and rejoining reactions that occur during transposition of mobile genetic elements called DNA transposons. Various organisms have occasionally utilized transposase genes to create new genes with beneficial cellular functions. This is called “domestication” of transposases. Intriguing instances of domestication of piggyBac transposases were found in the ciliate *Paramecium tetraurelia*.

The PiggyMac (Pgm) domesticated transposase, which carries a potentially active catalytic site (1), is required during the sexual cycle of this unicellular eukaryote (Fig. A) for the precise elimination of ≈45,000 short non-coding Internal Eliminated Sequences (IESs) from the polyploid somatic macronucleus (Fig. B). IESs are eliminated through a precise cut-and-close process, during which Pgm cleaves double-stranded DNA at flanking TA dinucleotides located at IES ends.

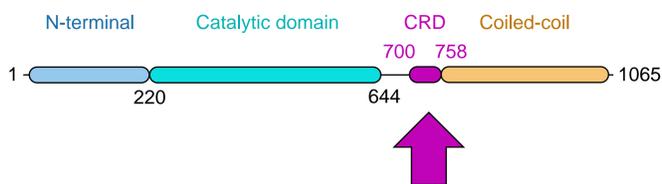
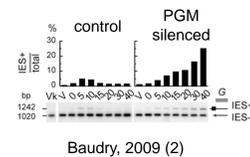


## A domesticated transposase precisely excises IESs

PiggyMac is necessary for Internal Eliminated Sequence (IES) excision

When the *PGM* gene is silenced by RNAi, the viability of *Paramecium* sexual progeny is strongly compromised due to the retention of all IESs that are normally excised in a wild-type control.

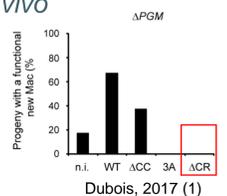
This shows that Pgm is necessary for the correct excision of the extraneous DNA.



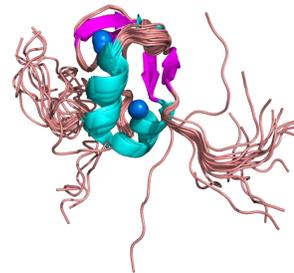
The cysteine-rich domain is essential for Pgm activity *in vivo*

The complementation assay in which PiggyMac is added to  $\Delta$ PGM cells, shows that if PiggyMac does not include its cysteine-rich domain (CRD), the progeny does not contain functional somatic nuclei.

Thus, the Pgm cysteine-rich domain is essential for the activity of Pgm in *P. tetraurelia*'s genome rearrangement.



## Characterization of the Cysteine-rich domain of PiggyMac



PiggyMac CRD

It forms an interlaced zinc-finger type structure



Diffusion NMR suggests that the CRD remains a monomer in solution

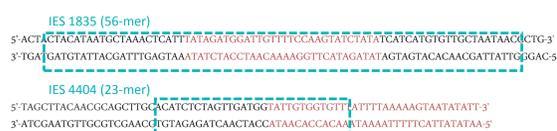
[Pgm CRD] (μM)	D (m <sup>2</sup> s <sup>-1</sup> )	Apparent MW (Da)
50	9.59 x 10 <sup>-11</sup>	11314
100	1.00 x 10 <sup>-10</sup>	9979
200	9.48 x 10 <sup>-11</sup>	11713
250	9.41 x 10 <sup>-11</sup>	11976

From the diffusion coefficient, we can estimate the molecular weight. The expected molecular weight for a monomer of PiggyMac CRD (with 2 Zn atoms) is 9787 Da.

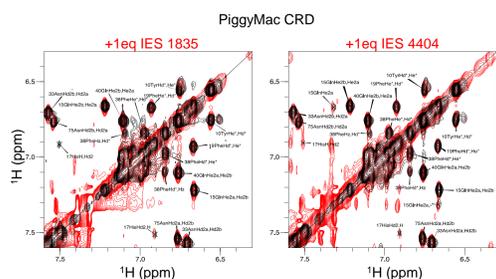
## Objective

Understand the role of the cysteine-rich domain in PiggyMac's transposase activity.

## PiggyMac CRD does not recognize IES DNA sequences



The DNA sequences (dashed lines) were taken from the *P. tetraurelia* genome at or adjacent to excision sites (dark red).

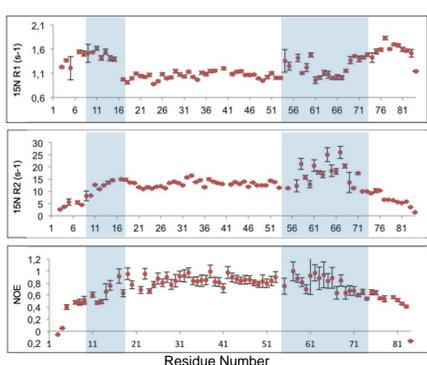


PiggyMac CRD does not appear to recognize DNA sequences.

Using two DNA sequences that either contained the entire eliminated sequence (IES 1835) or is upstream (IES 4404) from the excision site (the cleavage site is expected to bind Pgm catalytic domain not the CRD) no changes were observed in the <sup>1</sup>H,<sup>1</sup>H-NOESY spectrum of Pgm CRD.

Unlike in PiggyBac (wild transposase from *Trichoplusia ni*) (3), the role of the CRD does not appear to be recognition of the cleavage site by its DNA target sequences

## An interaction region displays peculiar relaxation behavior

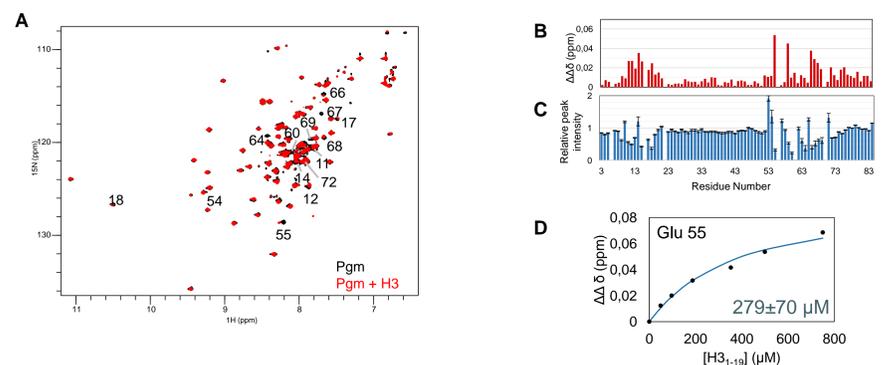


NMR relaxation measurements of Pgm CRD.

<sup>15</sup>N R<sub>1</sub>, R<sub>2</sub> and heteronuclear <sup>15</sup>N-<sup>1</sup>H NOE relaxation parameters measured at 950 MHz and 293 K.

The interaction regions identified to interact with H3 are highlighted in blue. The region corresponding to residues 57-71 displays increased R<sub>2</sub>, possibly due to exchange in the μs to ms timescale.

## PiggyMac CRD interacts with histone H3 N-terminal tail

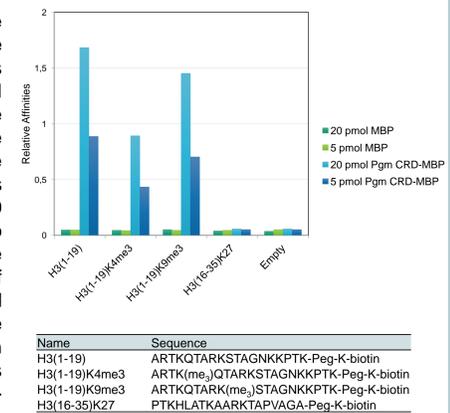


Histone H3 interacts with two regions of PiggyMac CRD.

A <sup>1</sup>H,<sup>15</sup>N-HSQC spectra (<sup>1</sup>H 800 MHz) of 100 μM <sup>15</sup>N-PiggyMac CRD in the absence (red) and presence of 1 mM histone H3[1-19] (black). B Chemical shift perturbations (Δδ) of amide resonances from PiggyMac CRD in spectrum A. C Relative peak intensity of PiggyMac CRD amide resonances, comparing the presence and absence of histone H3. D A dissociation constant of 279±70 μM was determined from the chemical shift perturbations. E Interaction zones of H3[1-19] shown in blue on PiggyMac CRD structure.

ELISA shows interaction between H3 peptides and Pgm CRD.

Biotinylated *P. tetraurelia* histone peptides (see table) were synthesized by Proteogenix. Each well on Pierce Streptavidin Coated High Capacity 96-well Plate was washed 3 times with 200 μL Wash Buffer (25mM Tris pH 7.4; 150mM NaCl; 0.1% BSA; 0.05% Tween 20). The biotinylated peptides (4 μM, in 100 μL Wash Buffer) were applied into wells to saturate their surface and the plate was incubated at room temperature for 1h. The plate was washed three times with 200 μL Wash Buffer and then 100 μL of purified MBP-CRD or MBP (40 nM) were added to each well. The plate was incubated at 4°C overnight before washing (as above) to remove unbound proteins. 100 μL of monoclonal anti-MBP-HRP antibody (NEB #8038S; diluted 1/10000 in Wash Buffer) was added to each well and the plate was incubated for 30 min at room temperature, then washed three times. Peroxidase enzyme activity was measured with tetramethylbenzidine (1 Step™ Ultra TMB-ELISA, ThermoScientific) using a TECAN Infinite 200 pro reader at 450 nm.



## Conclusions

NMR and ELISA experiments suggest that the CRD may play a role in identifying IES excision sites via the recognition of the histone H3 tail.

Further investigations are needed to understand if there is a preference for the other histones (H1, H2, H4) and/or certain histone modifications.

(1) Dubois E, Matthy N, Régnier V, Bischerour J, Baudry C, Trouslard R and Bétermier M. Multimerization properties of PiggyMac, a domesticated piggyback transposase involved in programmed genome rearrangements. *Nucleic Acid Research* (2017), 45(6):3204-3216.  
 (2) Baudry C, Malinsky S, Restituto M, Kapusta A, Rosa S, Meyer E, Bétermier M. PiggyMac, a domesticated piggyBac transposase involved in programmed genome rearrangements in the ciliate *Paramecium tetraurelia*. *Genes & Development* (2009), 23(21):2478-2483.  
 (3) Morellet N, Li X, Wieninger S A, Taylor J L, Bischerour J, Moriau S, Lescop E, Bardiaux B, Matthy N, Assrir N, Bétermier M, Nilges M, Hickman A B, Dyda F, Craig N L, Guittet E. Sequence-specific DNA binding activity of the cross-brace zinc finger motif of the piggyBac transposase. *Nucleic Acids Research* (2018) 46(5):2660-2677.