One for all, and all for one: PiggyMac-Like(s) and PiggyMac involved in IES elimination during programmed genome rearrangements in Paramecium tetraurelia.

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Résumé

The germline genome of ciliates undergoes extensive rearrangements following sexual events, during the development of a new somatic macronucleus from the germline micronucleus. In Paramecium, genome rearrangements include the precise excision of numerous single-copy Internal Eliminated Sequences (IESs) from the somatic DNA. These rearrangements have been described as a "cut and close" mechanism related to DNA transposition. They are initiated by DNA double strands breaks that require the activity of a domesticated piggyBac transposase, PiggyMac. "Cut and paste" DNA transposons generally harbour specific DNA sequences at their extremities that allow transposases to specifically bind and cleave DNA. In contrast, Paramecium IESs are short DNA sequences, unrelated to piggyBac transposons and devoid of any significant consensus sequence. One major issue is how the unique PiggyMac enzyme can precisely target all IESs. A hidden Markov model (HMM)-based search for the piggyBac transposase catalytic domain in the updated protein database from P. tetraurelia led to the identification of nine additional piggyBac-related proteins encoded by the MAC genome. Since these proteins share structural homology with Pgm, we named them PiggyMac-Like (PgmL1-9)). Several PGML genes are paralogs from the last and intermediate whole genome duplications that occurred during Paramecium evolution. For this reason, we grouped them into five different families (PGML-1, PGML-2, PGML4&5, PGML6&7 and PGML3,8&9). RNA sequencing data, from cells undergoing the sexual process of autogamy, shows that all PGML genes are specifically expressed during development of the new somatic macronucleus. Systematic RNAi-mediated gene silencing of all PGMLs demonstrated that each family is essential to eliminate IESs and recover a viable progeny. Unlike Pgm, we failed to map a canonical DDD catalytic triad in the putative catalytic domain of PgmLs, suggesting that PgmLs are catalytically inactive. Thanks to a heterologous protein expression system, we demonstrated that PgmLs are direct partners of Pgm. These data, together with a genome-wide survey of IES retention in cells silenced for individual PGML families, allow us to propose a model for PgmL action in IES excision.

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