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Progeny of germ line knockouts of *ASI2*, a gene encoding a putative signal transduction receptor in *Tetrahymena thermophila*, fail to make the transition from sexual reproduction to vegetative growth

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Abstract

The ciliated protozoan *Tetrahymena* has two nuclei: a germ line micronucleus and a somatic macronucleus. The transcriptionally active macronucleus has about 50 copies of each chromosome. At sexual reproduction (conjugation), the parental macronucleus is degraded and new macronucleus develops from a mitotic product of the zygotic micronucleus. Development of the macronucleus involves massive genome remodeling, including deletion of about 6000 specific internal eliminated sequences (IES) and multiple rounds of DNA replication. A gene encoding a putative signal transduction receptor, *ASI2*, (anlagen stage induced 2) is up-regulated during development of the new macronuclei (anlagen). Macronuclear *ASI2* is nonessential for vegetative growth. Homozygous *ASI2* germ line knockout cells with wild type parental macronuclei proceed through mating but arrest at late macronuclear anlagen development and die before the first post-conjugation fission. IES elimination occurs in these cells. Two rounds of postzygotic DNA replication occur normally in progeny of *ASI2* germ line knockouts, but endoreduplication of the macronuclear genome is arrested. The germ line *ASI2* null phenotype is rescued in a mating of a knockout strain with wild type cells.

Keywords: ASI2; Cache domain; DNA replication; signal transduction receptor; *Tetrahymena thermophila*

Introduction

Tetrahymena thermophila is a unicellular eukaryote, with two nuclei (reviewed in Karrer, 2000). The germ line micronucleus is diploid and transcriptionally silent in vegetatively dividing cells. The somatic macronucleus contains about 50 copies of each macronuclear chromosome. It is transcriptionally active, and responsible for the phenotype of vegetatively growing cells.

In rich medium, *Tetrahymena* cells reproduce by asexual fission, during which the micronucleus divides mitotically and the macronucleus divides amitotically. That is, there are no functional centromeres in the macronucleus and macronuclear alleles are distributed to asexual progeny at random (reviewed in Frankel, 2000).

Under conditions of starvation, *Tetrahymena* cells of different mating types pair and initiate sexual reproduction, which is called conjugation (Fig. 1). The micronucleus undergoes meiosis. Three of the meiotic products degenerate and the fourth undergoes prezygotic mitosis. Mating cells exchange pronuclei and fertilization occurs when the migratory nucleus from the mating partner fuses with the stationary pronucleus. The zygotic nuclei undergo two postzygotic mitoses to produce four nuclei in each cell, two of which develop into new macronuclei (macronuclear anlagen) and two into new micronuclei. The parental macronucleus is degraded apoptotically. Finally, one of the new micronuclei degenerates and the other divides mitotically, initiating the first vegetative cell division (Reviewed in Karrer, 2000).

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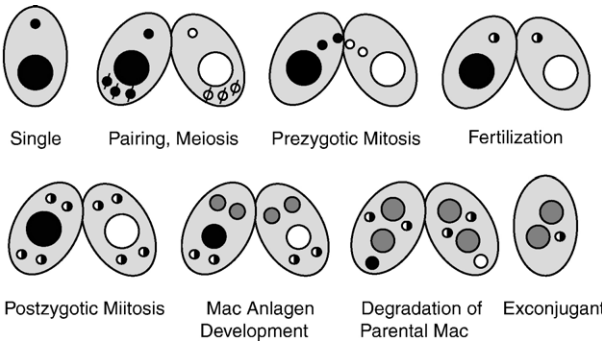

Fig. 1. Sexual reproduction in *Tetrahymena*. Black and white indicate two different alleles of a gene. Gray represents mixed alleles in the macronuclear anlagen.

One of the key events in the development of the macronuclear anlagen is the deletion of approximately 6000 specific sequences from the macronuclear genome. In most cases this deletion is accompanied by the ligation of flanking sequences, and thus the elements are referred to as internal eliminated sequences (IES). The protein Pdd1p (for programmed DNA degradation) is required for IES elimination (Coyne et al., 1999), which occurs via a RNAi-like mechanism (Mochizuki et al., 2002; Yao et al., 2003). Pdd1p appears early in conjugation and first accumulates in the parental macronucleus, where it is thought to be associated with small RNAs (scRNA) generated by a dicer-like protein (Mochizuki and Gorovsky, 2005; Malone et al., 2005). Subsequently, Pdd1p is concentrated in the "conjusome", a non-membrane bound, electron-dense particle in the anterior cytoplasm of postzygotic pairs, (Janetopoulos et al., 1999). Eventually, Pdd1p is transferred to the macronuclear anlagen. Staining with anti-Pdd1p reveals a punctate pattern showing colocalization of Pdd1p with the IES (Madireddi et al., 1996). This is thought to establish a specialized chromatin structure, further characterized by methylation of histone H3 at the K9 residue (Liu et al., 2004), that is required for DNA elimination.

Much of sexual reproduction is driven by transcription in the parental macronucleus. Several genes have been shown to be up-regulated during meiosis, before the new macronucleus develops (Martindale and Bruns, 1983). Four genes involved in excision of the IES, PDD1, PDD2, TWI1, and DCL1, are required in the parental macronucleus. Cells that are somatic knockouts of those genes do not complete DNA rearrangement and fail to make viable progeny (Coyne et al., 1999; Mochizuki and Gorovsky, 2005; Nikiforov et al., 1999). PDD1 is also transcribed in the macronuclear anlagen. The function of the zygotic Pdd1p, if any, is unknown.

The present study describes a gene, *ASI2* (*a*nlagen *s*tage *i*nduced gene 2) encoding a putative signal transduction receptor. As its name implies, the abundance of *ASI2* mRNA peaks at 9 h of mating, early in macronuclear anlagen development. *ASI2* is required in the macronuclear anlagen for sexual reproduction. Cytological analysis of matings between germ line *ASI2* knockouts shows the progeny develop new macronuclei, the parental macronuclei degenerate, and the cells separate to produce exconjugants. The molecular events of macronuclear anlagen development leading up to and including IES excision occur normally. However, endoreduplication of DNA in the macronuclear anlagen arrests in the early stages and progeny die prior to the first vegetative fission.

Materials and methods

Cell culture

Tetrahymena thermophila cultures were maintained in 1% or 2% PPYS (proteose peptone, yeast extract, and sequestrene) at 30°C (Orias et al., 1999).

Construction of Tetrahymena ASI2 knockout lines

Tetrahymena knockout strains were obtained by biolistic transformation with the BioRad Particle Delivery System (Cassidy-Hanley et al., 1997). An *ASI2* knockout construct was made, containing the neo2 cassette (Nikiforov et al., 1999) flanked by approximately 1 kb of genomic DNA 5′ and 3′ to *ASI2*. Cells from a mating between strains CU428 (VII) and B2086 (II) were bombarded at the crescent micronucleus stage. Transformants, in which the *ASI2* gene was replaced with the *neo2* cassette, were selected on the basis of resistance to paromomycin. Two types of transformants were obtained. Somatic (macronuclear) transformants resulted from bombardment of unpaired cells in the mating. A single germ line (micronuclear) transformant was identified as a progeny of the mating on the basis of 6-methylpurine resistance and subsequently confirmed as a germ line *ASI2* knockout. (The genotypes and phenotypes of all strains used in this study are provided in Table 1).

Northern blots

4–5 μg poly(A) mRNA isolated from *Tetrahymena* at different stages of conjugation, and from log phase and starving cells. RNA samples were treated and run on formaldehyde gels according to established protocols (Sambrook et al., 1989). The RNA was transferred to GeneScreen Plus nylon membrane (NEN) with 20× SSC for about 4 h. A lane containing 5 μg of RNA marker (Promega) was cut from the filter and stained with 0.04% methylene blue in 0.5 M sodium acetate pH 5.0 for 15 min, then de-stained for about 10 min in DEPC-treated dH_2O.

Table 1
Genotypes and phenotypes of *T. thermophila* strains

Strain	Micronuclear genotype	Macronuclear genotype	Phenotype
CU427	*chx1-1/chx1-1*	*CHX1*	cy-s, VI
CU428	*mpr1-1/mpr1-1*	*MPR1*	mp-s, VII
B2086	Wild type	Wild type	cy-s, mp-s, II
B*7	Star	Wild type	cy-s, mp-s, VII
MU114	*ASI2*/*asi2*: :neo, *CHX1/CHX1, mpr1-1/MPR*	*asi2*: :neo, *mpr 1-1*	pm-r, mpr-r, II
MU119	*asi2*: :*neo*/asi2: :neo, *CHX1/CHX1, MPR1,MPR1*	*asi2*: :neo, *mpr1-1*	pm-r, mp-r, II
MU120	*ASI2*/*ASI2*, *CHX1/CHX1*	*asi2*: :neo, *mpr1-1*	pm-r, mp-r, II
MU121.1	*asi2*: :neo/*asi2*: :neo, *chx1-1/chx1-1*, *MPR/MPR*	*asi2*: :neo	pm-s, cy-s, other than II, VI, VII
MU121.3	*asi2*: :neo/*asi2*: :neo, *chx1-1/chx1-1*, *MPR/MPR*	*ASI2*	pm-s, cy-s, other than II, VI, VII

Macronuclear phenotype designations: -r: resistant, -s: sensitive. Phenotypes of mutant genes are as follows: *mpr1-1*: 6-methylpurine (mp) resistant; *chx1-1*: cycloheximide (cy) resistant; : *Asi2*: :*neo*, paromomycin (pm) resistant. Mating types are designated by Roman numerals.