Gene 542 (2014) 221–231

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Identification of Tf1 integration events in *S. pombe* under nonselective conditions

Kristina E. Cherry, Willis E. Hearn, Osborne Y.K. Seshie, Teresa L. Singleton *

Winston Salem State University, 601 Martin Luther King Jr. Drive, WBA Science Building, Winston-Salem NC, USA

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form 11 March 2014
Accepted 14 March 2014
Available online 25 March 2014

Keywords:
Retrotransposon
Integration
Heterochromatin
Fission yeast
Trichostatin A

ABSTRACT

Integration of retroviral elements into the host genome is a phenomena observed among many classes of retroviruses. Much information concerning the integration of retroviral elements has been documented based on *in vitro* analysis or expression of selectable markers. To identify possible Tf1 integration events within silent regions of the *Schizosaccharomyces pombe* genome, we focused on performing an *in vivo* genome-wide analysis of Tf1 integration events from the nonselective phase of the retrotransposition assay. We analyzed 1000 individual colonies streaked from four independent Tf1 transposed patches under *nonselection conditions*. Our analysis detected a population of $G418^S/neo^+$ Tf1 integration events that would have been overlooked during the *selective phase* of the assay. Further RNA analysis from the $G418^S/neo^+$ clones revealed 50% of clones expressing the *neo* selectable marker. Our data reveals Tf1's ability to insert within silent regions of *S. pombe*'s genome.

1. Introduction

Long terminal repeat (LTR) retrotransposons are retrovirus-like transposons that replicate by reverse transcription of an RNA intermediate producing cDNA. The life cycle is similar to some higher order retroviruses in that the final stage of the life cycle includes insertion of the cDNA – using a viral encoded integrase protein – into the host chromatin (Boeke and Devine, 1998; Koonin et al., 2006). Because the eukaryotic cell chromatin is composed of euchromatic (regions of the chromatin that contains actively transcribing genes) and heterochromatic (regions of the chromatin referred to as "silent" for the transcription of genes) regions, careful target selection for integration is critical for the propagation of the virus and the survival of the host.

Integration of retroviral elements into the host genome is a phenomena observed among many classes of retroviruses. Much information concerning the integration of retroviral elements has been documented based on *in vitro* analysis or expression of selectable markers (Asante-Appiah and Skalka, 1997). The epigenetic environment of the eukaryotic cell's genome creates many challenges for target site selection. In order to fully analyze the cellular interactions of target site selection among retrotransposons it is critical to observe the impact of integration within the host environment.

Tf1 is an endogenous and active LTR retrotransposon of the fission yeast *Schizosaccharomyces pombe*. Tf1 contains a single open reading frame (ORF) that encodes 1331 amino acids. The ORF is composed of capsid, protease, reverse transcriptase, and integrase proteins (Hizi and Levin, 2005; Levin et al., 1993). As a member of the *Metavirus* genus (formerly called Ty3/Gypsy family) (Jern et al., 2005; Malik and Eickbush, 1999) the integrase protein contains a conserved module called a chromodomain (CHD) located within the carboxyl terminal (C-terminal) domain of the protein. Chromodomains are found in several eukaryotic proteins. The regions are believed to be essential for the methylation of histones as well as the interaction with heterochromatic regions (Eissenberg, 2001). The presence of a CHD region within the C-terminal domain of Tf1 integrase suggests a possible interaction with heterochromatic regions. Previous work has shown that mutations within the CHD of Tf1 affect transposition (Chatterjee et al., 2009).

Introduction of an *in vivo* assay system to monitor the life cycle of retroviral elements in prokaryotic (Naumann and Reznikoff, 2002) and eukaryotic (Boeke and Corces, 1989; Boeke et al., 1985; Curcio and Garfinkel, 1991; Jensen and Heidmann, 1991; Sandmeyer et al., 1990; Yang et al., 2007) cells has greatly advanced the field of retrovirology. Retrotransposition assays allow phenotypic monitoring of retroviral integrations *de novo*. The ability to isolate retroviral insertions is based on the cell's ability to express a selectable marker within the genome. The presence of a selectable marker could create a selection bias based on integrations into genomic regions that could suppress transcription (Francis and Spiker, 2005). The retrotransposon assay used to monitor the life cycle of Tf1 consists mainly of two phases. Phase I is a *nonselective phase* that selects against the donor plasmid carrying a copy of Tf1 (Levin and Boeke, 1992). Phase II is the *selective phase* that selects for genomic integrated copies of Tf1 based entirely on the

Abbreviations: TSA, trichostatin A; HDAC, histone deacetylase; IPCR, inverse polymerase chain reaction; G418, geneticin; *neo*, neomycin.

* Corresponding author at: Department of Life Science, WBA Science Building, Room 406, Winston-Salem State University, Winston-Salem NC 27110, USA.
E-mail addresses: kriswilliams1234@gmail.com (K.E. Cherry), willishearn19@gmail.com (W.E. Hearn), oseshie111@rams.wssu.edu (O.Y.K. Seshie), singletont@wssu.edu (T.L. Singleton).

http://dx.doi.org/10.1016/j.gene.2014.03.030
0378-1119/

expression of a selectable neomycin (*neo*) marker tagged to Tf1. Because Tf1 elements have never been observed to integrate into heterochromatic regions (Behrens et al., 2000; Singleton and Levin, 2002) it is unknown if a population of cells containing genomic Tf1 insertions exists within silent regions of the genome. Integration events within heterochromatic regions would go undetected in the *selective phase* of the retrotransposon assay. Previous reports have demonstrated this selection bias with T-DNA in the genome of *Arabidopsis* (Francis and Spiker, 2005; Kim et al., 2007; Gelvin, 2012).

Because Tf1 has been reported to harbor a chromodomain located within the C-terminal of the integrase protein, and because it is well documented that chromodomains are known to interact with heterochromatic regions of the genome, we anticipate possible Tf1 targeting to heterochromatic regions of *S. pombe*. Previous reports have isolated Tf1 insertions from the *nonselective phase* but bacteria were used to identify the insertion events (Singleton and Levin, 2002). In this study we report an *in vivo* genome-wide analysis of Tf1 transposition events. To identify possible Tf1 integration events within silent regions of the genome we focused on performing an *in vivo* analysis of Tf1 integration events from the nonselective phase of the retrotransposition assay. Our analysis detected a population of $G418^S/neo^+$ Tf1 integration events that would have been overlooked during the *selective phase* of the assay. Further RNA analysis from the $G418^S/neo^+$ clones revealed the expression of the *neo* selectable maker. Because silencing in *S. pombe* has an essential role in the formation of heterochromatin at the centromeres, telomeres, the mating-type locus, and ribosomal DNA (rDNA) (Hansen et al., 2005), the use of this organism in understanding how cells may silence viral infections is critical in advancing the design of therapies used to treat viral infections and diseases in mammals and humans.

2. Materials and methods

2.1. Media

S. pombe liquid and agar plate media were composed according to Singleton and Levin, 2002. Plasmid propagation plates were composed of Edinburgh Minimal Medium (EMM) (Forsburg and Rhind, 2006) lacking uracil and supplemented with appropriate amino acid, and Vitamin B1 (Thiamine) to suppress the *nmtl* promoter. Nonselective plates were composed of EMM supplemented with 1 mg/ml 5-fluoroorotic acid (5-FOA) (Boeke et al., 1987) (Zymo Research) and uracil at a final concentration of 50 μg/ml. Selective plates consisted of YES supplemented with 500 μg/ml geneticin (G418) (Agilent) and 1 mg/ml 5-FOA (YES/G418/5-FOA).

2.2. Construction of strains

The yeast strains and plasmids used in this study are described in Table 1. Yeast strain YHL6488 is a diploid *S. pombe* strain carrying the Tf1-ori/*neo* donor plasmid pTS1559-9 (Singleton and Levin, 2002). *S. pombe* strain YHL1282 is a haploid stain carrying the Tf1-*neo* donor plasmid pHL449-1 (Levin, 1995). *S pombe* strain YTS7078 is a haploid strain carrying the Tf1-ori/*neo* donor plasmid pTS1559-9.

2.3. Tf1 transposition assay

The Tf1 retrotransposition assay was performed as described previously (Singleton and Levin, 2002). Individual colonies were selected and used to grow patches on EMM lacking uracil supplemented with Vitamin B1 agar plates for 3 days at 30 °C. Patches were replica-printed to EMM agar plates lacking uracil and Vitamin B1 to induce transcription of Tf1. Patches were grown for 4 days at 30 °C then replica-printed to EMM agar plates supplemented with uracil and 5-FOA and allowed to grow for 3 days. This *nonselective phase* only allowed selection against cells containing the Tf1-*neo* and Tf1-ori/*neo* donor plasmids. Next, a *selective phase* consisted of replica-printing patches to YES/G418/5-FOA agar plates. Confluent cell growth was indicative of expression of the *neo* gene thereby creating G418-resistant ($G418^R$) colonies. The lack of cells' growth on YES-G418 is due to sensitivity to G418, and is G418-sensitive ($G418^S$).

2.3.1. Phenotypic analysis of Tf1 transposed colonies

Tf1 transposed patches (YTS6716, YTS6932, YTS7084, and YTS7133) from the nonselective phase of the assay were streaked to obtain single colonies. Two hundred-fifty (250) individual colonies were patched on EMM agar plates supplemented with uracil, Vitamin B1, and 5-FOA. Patches were replica-printed to YES agar plates containing G418 to determine the number of $G418^S$ and $G418^R$ colonies in the

Table 1
Yeast strains and plasmids used in this study.

	Genotype	Parent/plasmid	Reference
Parental yeast strains			
972	Wild type		ATCC[a]
YHL912	*h- ura4-294 leu1-32*		Singleton and Levin (2002)
YHL5661	*Stable diploid ura4-D18/ura4-D18 1eu1-32::nmt1-1acZ-1eu1-32::nmt-1acZ-1eu1 ade6-M210/ade6-M216*		Singleton and Levin (2002)
Nontransposed yeast strains			
YHL1282		YHL912/pHL449-1	ATCC[a]
YTS7078		YHL912/pTS1559-9	This study
YHL6488		YHL5661/pTS1559-9	This study
Transposed yeast strains			
YTS6716[b]		YHL5661/pTS1559-9	This study
YTS6932[b]		YHL912/pHL449-1	This study
YTS7084[c]		YHL912/pTS1559-9	This study
YTS7133[b]		YHL912/pTS1559-9	This study
Plasmid			
pTS1559-9	Tf1-ori/*neo*		Singleton and Levin (2002)
pHL449-1	Tf1-*neoAI*		Levin (1995)

[a] American type culture collection.
[b] Transposition using solid media.
[c] Transposition using liquid media.