Mutation Research 705 (2010) 205–216

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

In vivo mutation analysis using the ΦX174 transgenic mouse and comparisons with other transgenes and endogenous genes

Carrie R. Valentine [a], Robert R. Delongchamp [b], Mason G. Pearce [a], Heather F. Rainey [a], Vasily N. Dobrovolsky [a], Heinrich V. Malling [c], Robert H. Heflich [a,*]

[a] Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, 3900 NCTR Road, HFT-120, Jefferson, AR 72079, United States
[b] Department of Epidemiology, University of Arkansas for Medical Sciences, College of Public Health, Little Rock, AR 72205, United States
[c] Mammalian Mutagenesis Group, Laboratory of Toxicology, Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, United States

ARTICLE INFO

Article history:
Received 10 February 2010
Received in revised form 24 June 2010
Accepted 2 July 2010
Available online 14 July 2010

Keywords:
Transgenic mice
Somatic cell mutation
Germ cell mutation
Mutation frequency
Single-burst analysis

ABSTRACT

The ΦX174 transgenic mouse was first developed as an *in vivo* Ames test, detecting base pair substitution (bps) at a single bp in a reversion assay. A forward mutational assay was also developed, which is a gain of function assay that also detects bps exclusively. Later work with both assays focused on establishing that a mutation was fixed *in vivo* using single-burst analysis: determining the number of mutant progeny virus from an electroporated cell by dividing the culture into aliquots before scoring mutants. We review results obtained from single-burst analysis, including testing the hypothesis that high mutant frequencies (MFs) of G:C to A:T mutation recovered by transgenic targets include significant numbers of unrepaired G:T mismatches. Comparison between the ΦX174 and *lacI* transgenes in mouse spleen indicates that the spontaneous bps mutation frequency per nucleotide (mf_n) is not significantly lower for ΦX174 than for *lacI*; the response to ENU is also comparable. For the *lacI* transgene, the spontaneous bps mf_n is highly age-dependent up to 12 weeks of age and the linear trend extrapolates at conception to a frequency close to the human bps mf_n per generation of 1.7×10^{-8}. Unexpectedly, we found that the *lacI* somatic (spleen) bps mf_n per cell division at early ages was estimated to be the same as for the human germ-line. The bps mf_n in bone marrow for the *gpt* transgene is comparable to spleen for the *lacI* and ΦX174 transgenes. We conclude that the G:C to A:T transition is characteristic of spontaneous *in vivo* mutation and that the MFs measured in these transgenes at early ages reflect the expected accumulation of *in vivo* mutation typical of endogenous mammalian mutation rates. However, spontaneous and induced mf_ns per nucleotide for the *cII* gene in spleen are 5–10 times higher than for these other transgenes.

Published by Elsevier B.V.

Contents

* Corresponding author. Tel.: +1 870 543 7493; fax: +1 870 543 7393.
E-mail address: robert.heflich@fda.hhs.gov (R.H. Heflich).

1383-5742/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.mrrev.2010.07.001

1. Introduction to the ΦX174 transgenic mouse

In the early 1980 s, one of us (HVM) proposed that a transgene could be used to study *in vivo* mutation, and that the transgenic system should be designed to avoid mutation at CpG sites and should produce information about the nature of the mutation without sequencing [1]. This proposal led to the eventual development of the ΦX174 transgenic mouse, which has both a reversion [2] and a forward mutation assay [3]. This review article summarizes the research that has been conducted based on this original idea. Although the ΦX174 transgenic mouse has not been adopted for routine *in vivo* mutation detection, its unique characteristics have proven valuable in testing some of the assumptions underlying mutation detection in other transgenes.

ΦX174 is a single-stranded DNA bacteriophage of *Escherichia coli* C. Its genome was the first ever to be completely sequenced [4] and contains 5386 bases in a circular DNA molecule. ΦX174*am3cs70* DNA was introduced into the genome of C57BL/6-J mice in 1993 [2]. The *am3* mutation (587G → A) is at the central base pair of the seventh codon of gene *E*, the gene encoding the ΦX174 lysis protein [5,6], and it results in a T**A**G stop codon. The reversion assay that was devised using this construct detects exclusively 3 possible substitutions at this A:T base pair [7]. Mutation at other surrounding bases does not produce reversion because of the overlapping, staggered reading frame with gene *D* (Fig. 1) [8]. The phenotype conferred by the *am3cs70* mutation is failure to lyse *E. coli C*, resulting in no plaque. The other mutation contained in the transgene, the cold-sensitive mutation *cs70*, is thought to be a point mutation in gene *F* [9], which is included in the published ΦX174 sequence [4]. This transgenic mouse is often referred to as the "Malling Mouse" after its senior developer [2]. Based on Southern blot analysis, it was initially estimated that 50 copies of the transgene per chromosome were present in the mouse genome, or 100 copies per cell for homozygous mice.

The recovery of the transgenome requires only competent *E. coli* cells and genomic mouse DNA that has been *PstI*-digested and then re-ligated. The small, double-stranded, circularized molecule recovered from the murine genome is physically analogous to a plasmid molecule and is transduced into competent *E. coli* cells by electroporation. This double-stranded, circular molecule functions as the replicative form of ΦX174 [5] when introduced into cells.

2. Working hypothesis—recovery of mutation fixed in bacteria

Most transgenic rodent mutation assays have higher spontaneous MFs than the endogenous *Hprt* gene (Sections 1.4.2.2 and 3.6.6 in [10]). High spontaneous mutant frequencies are still considered a primary disadvantage of transgenic rodent mutation assay systems [11]. Since a large proportion of these transgene mutations are G:C → A:T at CpG dinucleotides, Skopek reasoned that mammalian methylation at CpG sites increased mutagenesis because of the higher G:C content of bacteria relative to mammalian cells [12,13]. However, redesigning the *lacI* target gene to have fewer CpG target sites (13 compared to 95) decreased this type of mutation to only 23% from 32% and did not reduce the overall MF. Instead, the spectrum of spontaneous mutations shifted from the N-terminus to the C-terminus of the gene. Another mechanism for the high proportion of G:C → A:T mutation in spontaneous spectra has been suggested by Holmquist to be CpG depletion targeted toward tandem repeats and mediated by RNA interference [14].

It was our working hypothesis that these elevated spontaneous MFs (as well as induced mutation) include mutation that is fixed in recovery bacteria, particularly from G:T mismatches in recovered DNA [15]. However, we show in this review that G:C to A:T mutation is characteristic of spontaneous *in vivo* mutation in the ΦX174 transgenic system and that typical spontaneous mutation frequencies of transgenes are not higher those of endogenous genes.

3. Recognition of mutation fixed in recovery bacteria

The importance of scoring mutations fixed only in the rodent tissue, as opposed to the recovery bacteria, was understood early in the development of transgenic mutation assays [16]. If damage

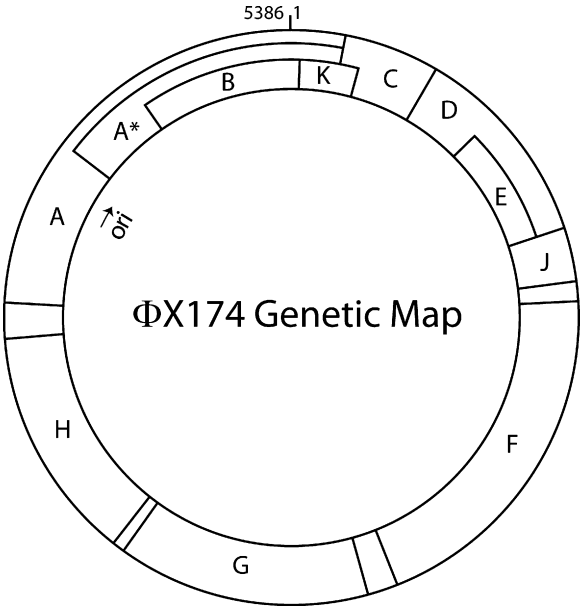

Fig. 1. Genetic Map of ΦX174. ΦX174 bacteriophage has a circular, single-stranded DNA genome with a correspondingly circular genetic map. The locations of the genes are shown, with their overlapping characters, relative to a unique *PstI* site at base 1 (map positions are based on [4]). Because of the circular genome, base 5386 is adjacent to base 1. The replication origin is designated "ori".