A a N -Ca a T a -S I G a b R Ta a / V

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Introduction

 $[4,5,6,7,\underline{8}]$, and evidence for its involvement in apparent in intermolecular transformation \mathbf{A} transformation \mathbf{Q} , although this is disputed for 10 , A key observation regarding these apparential theory splitting events is that they occur between \mathbf{A}, \mathbf{A} splitting that often short short homologous sequences. This presumably sequences. This presumably

Figure 2. An in system for the analysis of apparent trans-splicing. HXK1 KRE29 Template switching events produced by Superscript II and AMV are indicated. B: Purified substrate RNAs. C: RT-PCR using primers complementary to

each RNA on three independent RT reactions (lanes 1–3), and a no RT control (Lanes 4–5). The template for the DNA control (lanes 6–7) was HeLa cDNA with restriction fragments encompassing the entire sequence of the substrate RNAs. Upper panel 35 cycles, other panels 25 cycles. D: PCR reactions performed as in C on cDNA produced with AMV reverse transcriptase. Upper panel 35 cycles; lower panels 25 cycles. doi:10.1371/journal.pone.0012271.gov.html

at a single locus can be readily reproduced on a purified temperature on \mathbf{I}_0 in vitro using reverse transfer transfer

 A_s introduced 1 intron, reverse transcription temperature did \mathcal{A} not alter the observance of sense-antisense fusions (Fig. 4A). In contrast, the prominent bands representing both types of the problem of splitter locus were not observed when \mathbf{A} . The single when \mathbf{A} were not observed when \mathbf{A} substituted for Superscript II, and \mathbf{C} although \mathbf{U} obtained, suggesting that some temperature some temperature switching \blacktriangle However, the abundance of the abundance of the set of the set of $\mathbf{1}_{\mathcal{A}}$ was too low for us too low for us too sequence, so we cannot rule out their arising from * CR misspriming. The fact that prominent template-switching events were $\frac{1}{N_{\rm A}}$ excludes the possibility that senseantisense RNAs are produced by T Δ NA polymerase during dur transcription and survive the gel extraction step. We think the case the case the case the case the case the case of \mathcal{L}_c they should be a model with similar effective by either \mathcal{A}_1

 U proposed mechanism for the formation of sense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-antisense-a fusions requires two RT enzymes to be active on the same RNA molecule. This will occur frequently if \mathbf{A} is the state transcription is real primed from random from random to be less common \mathbf{A}_max is expected to be less common when $\mathbf{A}^{\top}(\mathcal{A}^{\prime})$ is used to prime synthesis from the poly \mathbf{A}^{\prime} test the effect of this change, $SPT7$ rNA was included with $A^{4,\bullet}$ in the presence or absence of E. coli poly(A) polymerase to add a $\mathbf{A}(\mathbf{A})$ to $\mathbf{A}^{(A)}$ then used in vitro for reverse were then used in vitro for reverse \mathbf{A} transcription as above but primed from \mathbf{A} or $\mathbf{C}^{(4)}$. This produced but produced but produced but \mathbf{A} the same pattern of products seen in previous experiments, which now depended on the presence of $A(A)$ polymerase (Fig. 4C). The problem of \mathbf{A} (a human matrix in the HeLa RNA) is presented to prove that RT efficiency was similar in the presence and absence of $\blacktriangle(A)$ polymerase. Note that some reverse reverse. transcription of the SPT7 \mathbb{R}^n NA still occurs in the absence of the absence of

 $T_{\rm eff} = \overline{\rm G}$

showing sites and observed sense-antisense functions. C: S PT7 substrate. D: S PT7 substrate. S PT7 substrate.

performed as 32 cycle PCR reaction, other panels shows a 32 cycles. E: SPT7 \sim SPT7 RNA showing primer binding sites and observed exon shuffling events. F: RT-PCR experiments performed as in d. Sequenced bands are indicated by *. doi:10.1371/journal.pone.0012271.gov.html

 $T_{\rm max}$ Generated by $G_{\rm max}$

 $\mathbf{A}(\mathbf{A})$ polymerase due to priming of the oligon \mathbf{A} (d \mathbf{A}) on short, encoded \blacktriangle (A) stretches in the substrate. The substrate show that \mathbf{t}_{c} . The same prime detected in random hexamer primed in random hexamer primed in random hexamer primed in \mathbf{t}_{c}

sense-antisense fusion, can also be generated as reverse transcriptase artifacts. Furthermore we present a simple test for identifying $\mathcal{A}_{\mathcal{A}}$ many template switching events based on comparison of MMLV

and $\mathbf{A} = \left(\mathbf{a}_{1}, \mathbf{a}_{2}, \mathbf{b}_{3}, \mathbf{c}_{4}, \ldots, \mathbf{c}_{n} \right)$

Materials and Methods

Substrates for in vitro assays assays \mathbf{A} and genomic \mathbf{A} and \mathbf{B} with Phusion (NEB) and cloned into pGEM-T (Promega). $\mathcal{O}(\lambda)$, λ is denoted were λ in the HXX1, E29 $F_{1/2}$ for $F_{2/2}$ for $F_{3/2}$ $F_{1/2}$ for $F_{2/2}$ for $F_{3/2}$ for $F_{3/2}$ specifies of $F_{3/2}$ \mathbf{A} oligonucleotides are given in \mathbf{A} . \mathbf{I}_1 \mathbf{I}_2 are linearized were linearized were linearized were linearized with \mathbf{A}_1 with Xhoi and 1 mg transcript using T7 NA polymeras (NEB) for 2 km at 37°C. Gels were run in 1x TBE, and \blacktriangle and \blacktriangle contained 8 M urea. Gels were started with \mathbf{B} and started with SY imaged using a Fuji FLA5100 scanner. RNA was eluted from acrylamide gel slices by crushing and soaking for 4 m in 0.5 M in 4 $N(A/1$ $ED^4 A/0.1\%$ D , M \mathbf{y} traction and extraction and ethanol precipitation $\mathbf{1}$ $\mathbf{\mu}$ and $\mathbf{\lambda}$ and $\mathbf{\mu}$ * Cr reactions on reverse transcribed material were performed material we with Phire (NEB), details of cycle number are given in individuals of cycle \mathcal{A} figure legends. Annealing temperature was \mathbf{A}_1 and \mathbf{A}_2 and \mathbf{A}_3 and \mathbf{A}_4 and \mathbf{A}_5 and \mathbf{A}_7 and \mathbf{A}_8 and \mathbf{A}_7 and \mathbf{A}_8 and \mathbf{A}_9 and \mathbf{A}_9 and \mathbf{A}_9 and \mathbf{A}_9 a HXK1/KRE29 °C and 53°C for SPT7 °C to -- -- -- 50 ng RNA was incubated with \mathbf{A} (A) \mathbf{A} polygraphy (NEB) and \mathbf{A} 1 $A^{\prime\prime}$, then cleaned on $A^{\prime\prime}$, then $A^{\prime\prime}$ columns ($A^{\prime\prime}$,). Superscript II 4 : 0.5 ng substrate RNA, 500 ng Hel $\,$ \rm{NA} (Invitrogen), 125 ng random hexamers and 0.5 ml 10 mM dNTPs in 6.5 μ l, total volume were denoted at 65°C for 5 min before μ 2 min on $2 \mu \lambda 5$ first strand buffer and $1 \mu \lambda = 0.1$ M D^max were added **in** \mathbf{u} is $\mathbf{0.5}$ $\mu\mathbf{\lambda}(100)$ under $(1, 1, 1, 1)$. reactions were incubated incubated incubated in 10 min at room temperature, $42^{\circ}\mathrm{C}$ for 50 min and 70° C for 15 min and $\blacklozenge(1)$ priming, 250 ng \mathbf{A} ol $\mathbf{C}^{(4)}$ 18 was added in place of hexamers, and reactions were reactions were reactions were reactions were reactions were reacted in \mathbf{A} . heated to $42^\circ\mathrm{C}$ prior to enzyme addition. Superscript III re-Fig. actions were performed as per manufacturer's instructions at the indicated temperatures. DNA temperatures. \mathbf{A}_{out} temperatures. DNA from 500 , with Δ Δ above, with Δ as above, Δ above, Δ X hoi-Pvui fragments of the template Λ individual plasmids. Where is the temperature $\begin{array}{llll} \mathrm{A}_{\mathrm{max}} & \mathrm{p}_{\mathrm{max}} & \mathrm{D} \left(\mathrm{C}_k \, \mathrm{A}_{\mathrm{max}} \right) & \mathrm{p}_{\mathrm{max}} & \mathrm{p}_{\mathrm{max}} & \mathrm{p}_{\mathrm{max}} \end{array}$ Δ solution. $A = \begin{bmatrix} 4 & 0.5 \end{bmatrix}$ $A, 500$ ng HeLa A (invitrogen) and A 125 , and random here in the second λ volume 8.25 μ_{∞} are heated 5 min at 70° and 5 min center at $1.25 \ \mu$ 10 mm s $\ \sqrt{37}$, $\frac{1}{2}$ and $2.5 \ \mu$ 5 buffer were added by $0.5 \mu \lambda (5)$ AMV (Promega). reactions were incubated for 1 h at $37^\circ\mathrm{C}.$

Supporting Information

- 10. Zhu S, Li W, Cao Z (2002) Does MMLV-RT lacking RNase H activity have the capability of switching templates during reverse transcription? FEBS Letters 520: 185–185. 11. Hastings KEM (2005) SL trans-splicing: easy come or easy go? Trends in genetics 21: 240–247. 12. Horiuchi T, Aigaki T (2006) Alternative trans-splicing: a novel mode of pre- mRNA processing. Biology of the cell 98: 135–140. 13. Herai RH, Yamagishi ME (2010) Detection of human interchromosomal trans-
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