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Identification of 3′-terminal Exons from Yeast Artificial Chromosomes

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We report an extension of 3′-terminal exon trapping technology to the identification of transcribed sequences from yeast artificial chromosomes (YACs). A 350-kb YAC containing mouse genomic DNA was gel-purified and used as the target DNA for the 3′-terminal exon trapping strategy. A novel direct ligation/transfection approach was employed to increase the efficiency of trapping 3′-terminal exons from recombinant vector-derived chimeric mRNA. The resulting RT–PCR product was then used to generate a plasmid library. Randomly chosen individual subclones from this library were sequenced, and the results indicate that 86% met sequence criteria characteristic of 3′-terminal exons, whereas 14% were background from identified sources. PCR mapping efforts suggest eight putative last exons present within this YAC, whereas RT–PCR studies demonstrate that three reside within valid expressed sequences.

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With the widespread use of yeast artificial chromosomes (YACs) in vertebrate genome mapping efforts, it becomes paramount to be able to identify genes that reside within these YACs. Many approaches have been developed to identify transcribed sequences from specific regions of the genome, yet most rely on either the presence of certain transcripts within cDNA populations or sequence knowledge of the genomic DNA.[1–5] Exon trapping offers a strategy for obtaining transcribed sequences directly from fragments of unsequenced genomic DNA, thus bypassing problems associated with direct cDNA screening and large-scale genomic sequencing.[6–10] Exon trapping has demonstrated its utility to gene identification from such sources of genomic DNA as plasmids, individual cosmids, and pooled cosmids.[10–12]

Two distinct approaches to exon trapping have been developed: Internal exon trapping and 3′-terminal exon trapping. 3′-Terminal exon trapping was reported as a method that offers several advantages over internal exon trapping, such as (1) a positive selection scheme, (2) the isolation of larger DNA fragments, and (3) the fact that the vast majority of genes contain a single 3′-terminal exon that makes further gene-mapping studies less confusing.[10]

Here, we report the application of 3′-terminal exon trapping to gene identification from a 350-kb mouse YAC, where we found that a large percentage of resulting subclones expected to contain 3′-terminal exons pass sequence analysis criteria indicative of valid last exons. Further mapping and gene expression studies demonstrate the usefulness of this approach to the isolation of valid 3′-terminal exons.

MATERIALS AND METHODS

DNA Preparations

High-molecular-weight DNA was purified as described[13,14] from YAC FAK-G3, which was isolated from the Princeton/MIT mouse genomic library.[15,16] The 350-kb YAC was separated on preparative pulsed-field gels made of 1% SeaPlaque agarose (FMC) and excised (apparently free of other yeast chromosomal DNA). The resulting gel slices were treated with Beta-agarase I (NEB) according to manufacturer's recommendations and the DNA was ethanol-precipitated as described.[17] Purified YAC DNA was digested to completion with *Eco*RI, phenol/chloroform-extracted, ethanol-precipitated, and resuspended in TE at a concentration of 500 ng/μl. The trapping vector pTAG4 was digested to completion with *Nru*I–*Eco*RI and electrophoresed on a 0.8% agarose gel to separate vector from the 300-bp polylinker fragment. The linear vector was extracted from the gel slice with glass-milk purification and resuspended at 500 ng/μl in TE. Equal amounts of YAC fragments (500 ng) and prepared pTAG4 (500 ng) were mixed in 1× ligation buffer (50 mM Tris-HCl at pH 7.6, 10 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, 5% PEG-8000) with T4 DNA ligase enzyme (1 unit) in a total volume of 5 μl to give a final DNA concentration of 200 μg/ml. The ligation reaction was allowed to incubate overnight at 15°C.

3′-Terminal Exon Trapping Protocol

In a novel application of increasing the

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efficiency of expressing trapped exons from recombinant vector, the ligation reaction was not subcloned in *E. coli*, rather it was directly transfected into 5×10^5 COS-7 cells that were seeded into a single well of a 6-well plate the day before transfection using Lipofectace according to the manufacturer's recommendations (GIBCO BRL). Poly(A)$^+$ mRNA was isolated from transfected COS-7 cells 16 hr later by the Micro-Fast Track system according to the manufacturer's protocol (Invitrogen). Poly(A)$^+$ RNA was quantitated, and 500 ng was mixed with 5 ng of the adapter primer (AP) and EDTA (final concentration of 20 mM) in a total volume of 20 μl. The AP primer sequence is 5'-AAGGATCCGTCGACATCGATAATACGAC$(T)_{17}$-3'. The mixture was heated to 70°C for 3 min followed by incubation for 5 min at 42°C at which time 30 μl of the reverse transcription mixture that was prewarmed to 42°C was added. The reverse transcription mixture consisted of 12 μl of H_2O, 10 μl of 5× reverse transcriptase (RT) buffer (250 mM Tris-HCl at pH 7.5, 15 mM $MgCl_2$, 250 mM KCl), 5 μl of 100 mM DTT, 1 μl of 25 mM dNTPs, and 2 μl (400 units) of Superscript II reverse transcriptase (GIBCO BRL). The reaction was allowed to incubate at 42°C for 30 min, at which time it was heated to 55° for 5 min and 1 μl (2.6 units) of RNase H (GIBCO BRL) was added, and the reaction was incubated at 55°C for an additional 15 min to degrade residual RNA. The RT enzyme was then heat-killed by incubation at 94°C for 10 min, and the cDNA pool was stored at −20°C until used for the PCR reaction. Five microliters of the cDNA pool was used as template for PCR #1, which consisted of a hot start PCR reaction with the primer set SV40P/UAP under the following conditions: 94°C for 3 min, 80°C for 1 min when 2.5 units of *Taq* polymerase was added, then cycled at 94°C for 30 sec, 55°C for 30 sec, 72°C for 2 min for 20 cycles, with a final extension at 72°C for 5 min. Standard PCR buffer was used with a final $MgCl_2$ concentration of 1.25 mM. The primer SV40P (5'-AGCTATTCCAGAAGTAGTGA-3') is specific for the portion of the cDNA that is coded for by the SV40 promoter of pTAG4, whereas UAP (universal amplification primer, 5'-CUACUACUACUAGTCGACATCGATAATACGAC-3') is specific for the tail adapter of the AP. A fraction (8 μl) of the product from PCR #1 was digested to completion with *Eco*RI, and 0.1 μl of the digestion reaction was used as template for PCR #2. This enzyme digestion step is used to remove PCR product generated from unspliced precursor RNA, thus decreasing background in the final analysis. PCR #2 was performed using hot start with the primer set Ad2/UAP. The reaction was heated to 94°C for 3 min and cooled to 80°C for 1 min, 2.5 units of *Taq* polymerase was added, and cycled at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 2 min for 30 cycles, with a final extension at 72°C for 5 min. Buffer conditions were the same as for PCR #1. The primer Ad2 (5'-CAUCAUCAUCAUCAGTACTCTTGGATCGGA-3') is specific for the second exon of pTAG4, whereas the use of UAP imparts a heminested approach to PCR #2 to give greater specificity to the reaction.

Subcloning and Sequencing

Product from PCR #2 was analyzed on a 1.2% agarose gel and used for UDG-mediated subcloning. The primers Ad2 and UAP contain UDG cloning tails that are designed to facilitate the directional subcloning of PCR product into the pAMP1 vector according to the manufacturer's recommendations (GIBCO BRL). PCR #2 product was shotgun cloned into pAMP1, *Escherichia coli* transformants were plated, and subclones were picked for double-stranded sequencing. Alkaline lysis double-stranded DNA preparations of individual subclones were prepared using the Wizard miniprep kit according to the manufacturer's recommendations (Promega). Dye–primer sequencing reactions using M13 forward and M13 reverse primers were performed with the Prizm cycle sequencing kit according to the manufacturer's recommendations (ABI). Sequence was analyzed on an ABI 373 automatic sequencer.

Gene Expression Protocol

Total RNA was isolated by Trizol reagent (GIBCO BRL) according to the manufacturer's recommendations. Five micrograms of total RNA from NIH-3T3 cells plus 5 μg of each of the following mouse tissues was pooled for use as the RT–PCR substrate: pancreas, kidney, heart, lung, thymus, brain, spleen, and blood. Reverse transcription was performed using 5 μg of the mixed RNA population using the AP to prime at poly(A) tails. Subsequently, 5 μl of the RT reaction was used for hot start PCR according to the following parameters: Buffer conditions, enzyme concentrations, and primer concentrations were as described above in 30 cycle reactions of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 2 min, followed by a final extension at 72°C for 5 min. Results were analyzed on a 3% agarose/ethidium bromide gel.

RESULTS

The YAC FAK-G3 containing 350 kb of mouse genomic DNA was separated from yeast DNA by pulsed-field gel electrophoresis. YAC DNA was digested to completion with the restriction enzyme *Eco*RI, whereas the 3'-terminal exon trapping vector pTAG4 was double digested to completion with the enzymes *Nru*I–*Eco*RI and gel purified. pTAG4 prepared in this manner generates a blunt-ended, nonligatible site upstream of the SV40 transcriptional promoter and a sticky end *Eco*RI site downstream of the second exon of pTAG4. *Eco*RI-digested YAC DNA was ligated to prepared pTAG4 followed by direct transfection of the ligated products into COS-7 cells to induce transient expression. The result of such a ligation is to form concatamers consisting of YAC *Eco*RI fragments flanked on both ends by pTAG4 that, when expressed in COS-7 cells, yield chimeric transcripts initiated from the SV40 promoter followed by RNA processing where the 5' splice site of vector exon 2 searches for a 3' splice site of any exon (internal or 3'-terminal) residing downstream in the target DNA. All biochemical processing of chimeric RNA transcripts terminates with 3'-terminal exons found within target DNA, and stable vector-initiated mRNA is generated.

The trapped 3'-terminal exons were amplified by the RT–PCR strategy as described, and results were analyzed on a 1.2% agarose gel as shown in Figure 1. Results indicate that a number of discrete bands were evident (lane 2) from ~250 to 800 bp. This material was directly cloned into the vector pAMP1 by UDG-mediated cloning, and multiple clones were picked at random for sequencing.

The following two criteria, based on sequence analysis, must be met to warrant further analysis of potential 3'-terminal exons. First, each potential exon

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FIGURE 1 Analysis of the 3′-terminal exon trapping result. Results were analyzed on a 1.2% agarose/ethidium bromide-stained gel. (Lane *1*) 100-bp ladder DNA marker with the band sizes noted (GIBCO BRL); (lane *2*) resulting PCR product of trapped sequences ranging from 220 to 800 bp.

Ad2

ttccagtactcttggatcggaaacccgtcggcctccgaacgGACGGAGATTAAAGTGTTGCACAGAAGCTGGTATGTTTGCAATCCGAGC
ACGGAGGAAAATTGCTACAGAGAAGGACTTCTTAGAAGCTGTAAATAAGGTCATCAAGTCTTACGCCAAATTCAGCGCTACT
CCCCGCTACATGACATACAATTGAGCCCCAGGGCTCTGAGTGAAAGAACTTCCTGTGCTTTTGTAACTTGTTGCCACCGCAC
AAAAATAAAAGATTTAAAAACTGaaaaaaaaaaaaaaaaaagtcgtattatcgatgtcgactg

UAP

FIGURE 2 Example of sequence analysis of PCR product. Clone 25 demonstrates the following hallmarks of a potential 3′-terminal exon. Novel sequence of 237 bp is shown in uppercase letters flanked by vector-derived sequence upstream and poly(A) tail/3′-RACE sequence downstream in lowercase letters. Sequences of the primers used for PCR (Ad2 and UAP) are indicated, the splice junction between exon 2 of the vector and novel sequence is indicated by the arrow, and the poly(A) signal (AATAAA) is underlined.

must have been derived from a splice event as evidenced by novel sequence directly following the splice junction of the 5′ splice site of vector exon 2. Second, the vast majority of potential exons will contain a consensus poly(A) signal (AATAAA or ATTAAA) 10–30 nucleotides upstream of the poly(A) tail. Sequence analysis indicates that 25 of 29 (86.3%) of the subclones passed both these criteria. These sequences represented 10 different potential 3′-terminal exons; thus, most were sequenced multiple times. Interestingly, 3 out of 29 sequences (10.3%) were apparently derived from COS-7 endogenous mRNA resulting from mispriming at the PCR level as evidenced by the fact that these clones were not derived from pTAG4. There was also a single clone derived from precursor RNA (3.4%) as evidenced by the failure to remove the intron between vector exon 2 and a YAC-derived 3′-terminal exon. An example of an analyzed sequence is shown in Figure 2. The novel sequence is printed in large script, whereas the vector-derived sequences and those used for 3′ rapid amplification of cDNA ends (RACE) amplification are in small script. Primers used for PCR are indicated, and the splice junction defining a splice event is shown by the black arrow. The poly(A) signal is also shown underlined.

Computer analyses of these sequences using the BLASTN program indicated clone 25 is the mouse homolog of the human MSS1/yeast YTA3 gene, which is a positive modulator of HIV Tat-mediated *trans*-activation.[18] Sequence homology indicates 90% conservation at the nucleotide level between bases 18 and 160 of clone 25 with the MSS1 gene and 70% conservation of these same bases with the YTA3 yeast homolog. Clone 5 shows the presence of a low abundant rodent repeat embedded within the sequence. All sequences contained multiple stop codons in all three frames, lending additional sequence evidence of 3′-terminal exon validity.

These 10 candidate 3′-terminal exons were further evaluated to establish their origin and validity as true exons. Sequence-specific oligonucleotides were used in PCR reactions to map each candidate exon to the FAK-G3 YAC, mouse genomic DNA, or yeast DNA. Results are shown in Figure 3. Clones 4, 5, 12, 19, 25, 44, and 55 were positively derived from the YAC as evidenced by the correctly sized PCR product from each clone in YAC plus yeast DNA lanes (Y + Ye) and mouse genomic DNA lanes (M) but not in yeast-only DNA lanes (Ye). Clones 43 and 71 were apparently derived from contaminating yeast DNA. Clone 74 has never given PCR product from any of these templates despite using multiple primers and PCR reactions at various conditions (data not shown). The possibility exists that its origin may not be detectable by PCR using these primers because an upstream exon that resides on the same (or possibly different) *Eco*RI fragment containing the last exon may have been simultaneously trapped. As a result, these two exons may be sufficiently separated in the genomic structure of the gene to disrupt PCR.

To establish the validity of these sequences as true 3′-terminal exons, gene expression studies were performed using RT–PCR. The same sequence-specific primers used for the above studies were also used in RT–PCR of RNA derived from eight different mouse tissues and NIH-3T3 cells. Results of the PCR reactions, as shown in Figure 4, demonstrate that clones 12, 19, and 25 are valid last exons as evidenced by the correct-size PCR product in cDNA generated from the mixed population RNA. PCR of RNA treated with (+) and without (−) re-

FIGURE 3 PCR mapping analysis. Sequence-specific primers were generated from each trapped sequence and used in PCR reactions to map their origin. Each primer set was used against three sources of template DNA: YAC+yeast (Y+Ye), yeast-only (Ye), and mouse genomic DNA (M). Clones 4, 5, 12, 19, 25, 44, and 55 were trapped from the purified YAC, whereas clones 43 and 71 were trapped from yeast-contaminating DNA.

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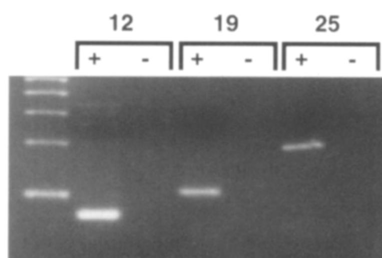

FIGURE 4 Result of gene expression study. The primer sets used for the genomic mapping of exons were utilized in RT–PCR analysis of mouse total RNA isolated from eight different tissues plus the mouse tissue culture cell line NIH-3T3. Results indicate that clones 12, 19, and 25 contain valid 3′-terminal exons as evidenced by PCR product in RT–PCR reactions containing RT (+) and not in reactions without RT (−). The −RT reaction is done as a negative control for DNA contamination of the RNA preparation.

verse transcriptase is performed to rule out DNA contamination of the RNA preparation used for the experiment. The other five potential exons did not give RT–PCR product in similar experiments. Clone 74 was also negative (data not shown).

DISCUSSION

The utilization of YACs as cloning vehicles has become a cornerstone of many genome mapping efforts; however, the ability to identify genes within those YACs has remained problematic. 3′-Terminal exon trapping was employed to establish its usefulness in identifying transcribed sequences from YACs. Results indicate the discovery of eight candidate last exons, of which three were validated, from a 350-kb YAC containing mammalian genomic DNA.

3′-Terminal exon trapping using YAC DNA as the target source offers certain advantages over existing gene identification technologies as well as some disadvantages. This approach utilizes unsequenced genomic DNA as the target source similar to direct selection and internal exon trapping; however, unlike direct selection, this approach does not rely on tissue-specific presence of particular cDNAs. Internal exon trapping has been applied to pools of cosmids containing equivalent genomic coverage of DNA as compared to YACs, and reports suggest that a single candidate exon is discovered every 20–80 kb of genomic DNA.[12] Results from this report indicate eight candidate last exons from 350 kb of genomic DNA rendering one exon (one gene) every 40–50 kb. This is in agreement with existing data using internal exon trapping; however, 3′-terminal exon trapping generally has a single target versus many internal exons as targets. Thus, these results suggest that 3′-terminal exon trapping is more efficient in gene identification than internal exon trapping.

The direct ligation/transfection method, as described in this paper, provides the ability to trap 3′-terminal exons from all restriction fragments on an equal basis regardless of size, complexity, or clonability of the target fragments. This is a direct advantage over internal exon trapping that relies on subcloning the target restriction fragments in *E. coli* before transfecting recombinants into COS-7 cells.

The end product of 3′-terminal exon trapping is a clone usually of 300–400 bp in length that represents only a fraction of the full-length cDNA. This is a disadvantage compared with direct selection that may yield near-full-length cDNAs. However, the size of the 3′-terminal exon compares favorably with the smaller clone size that results from internal exon trapping. Furthermore, the 3′-terminal exon of a gene consists mostly of the 3′-untranslated region (3′ UTR), which tends to be more gene specific. This may have the advantage of avoiding the protein-coding region of cDNAs when screening libraries, thus making the screen more specific. However, a drawback to the use of 3′-terminal exons for cDNA screening is the presence of repeats such as human Alu and LINE elements that reside within 3′ UTRs. Current estimates suggest that 10% of trapped 3′-terminal exons from human DNA contain a variation of one of these repeats.

Results from this report indicate that yeast sequences are also trapped along with YAC-derived sequences. This is a background problem that can be kept to a minimum by tedious gel purification of the YAC. However, at present, this problem cannot be entirely overcome, and its existence should be kept in mind.

Proving exon validity is also a problem that must be addressed. The standard approaches include using the subcloned potential exons as probes on Northern blots, in RNase protection experiments, and in screening cDNA libraries. An alternative approach uses sequence-specific primers in RT–PCR expression studies as reported here. The failure to validate five of eight clones as valid exons (from this paper) may result from several problems. First, these clones may be the product of cryptic activation leading to splicing of nonexonic DNA that is reasonably close to true exons in sequence. However, previous studies suggest that this should happen at low frequencies.[10] Second, the failure of RT–PCR studies to demonstrate expression may be the result of low relative abundancy of particular mRNA species in the tissues studied, suggesting that a battery of techniques needs to be pursued to show expression. Third, these clones may have been derived from unprocessed pseudogenes. Fourth, these sequences may reside within genes that are transcribed only at particular developmental stages and only in specific tissues. Finally, an event may exist where an internal exon from one gene may have been trapped along with the last exon from another gene. In this case, if primers are designed from each of the two exons, the PCR reaction will fail.

This paper demonstrates the usefulness of the 3′-terminal exon trapping strategy to the identification of transcribed sequences from YACs. All of the techniques used in this approach are standard molecular biology techniques from the YAC purification to gene expression studies. Results indicate that eight candidate 3′-terminal exons were cloned with three proven to be authentic by RT–PCR expression studies.

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REFERENCES

1. Parimoo, S., R. Kolluri, and S.M. Weissman. 1993. cDNA selection from total yeast DNA containing YACs. *Nucleic Acids Res.* **21:** 4422–4423.
2. Lovett, M. 1994. Fishing for complements: Finding genes by direct selection.

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Trends Genet. **10:** 352–357.

3. Liu, P., R. Legerski, and M.J. Siciliano. 1989. Isolation of human transcribed sequences from human-rodent somatic cell hybrids. *Science* **246:** 813–815.
4. Ubebacher, E.C. and R.J. Mural. 1991. *Proc. Natl. Acad. Sci.* **88:** 11261–11265.
5. Hochgeschwender, U. 1992. Toward a transcriptional map of the human genome. *Trends Genet.* **8:** 41–44.
6. Duyk, G.M., S. Kim, R.M. Myers, and D.R. Cox. 1990. Exon trapping: A genetic screen to identify candidate transcribed sequences in cloned mammalian genomic DNA. *Proc. Natl. Acad. Sci.* **87:** 8995–8999.
7. Auch, D. and M. Reth. 1991. Exon trap cloning: Using PCR to rapidly detect and clone exons from genomic DNA fragments. *Nucleic Acids Res.* **18:** 6743–6744.
8. Buckler, A.J., D.D. Chang, S.L. Graw, J.D. Brook, D.A. Haber, P.A. Sharp, and D.E. Housman. 1991. Exon amplification: A strategy to isolate mammalian genes based on RNA splicing. *Proc. Natl. Acad. Sci.* **88:** 4005–4009.
9. Hamaguchi, M., H. Sakamoto, H. Tsuruta, H. Sasaki, M. Tetsuichiro, T. Sugimura, and M. Terada. 1992. Establishment of a highly sensitive and specific exon-trapping system. *Proc. Natl. Acad. Sci.* **89:** 9779–9783.
10. Krizman, D.B. and S.M. Berget. 1993. Efficient selection of 3′ terminal exons from vertebrate DNA. *Nucleic Acids Res.* **21:** 5198–5202.
11. Huntington Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's Disease chromosomes. *Cell* **72:** 971–983.
12. Church, D.M., C.J. Stotler, J.L. Rutter, J.R. Murrell, J.A. Trofatter, and A.J. Buckler. 1994. Isolation of genes from complex sources of mammalian genomic DNA using exon amplification. *Nature Genet.* **6:** 98–105.
13. Anand, R., A. Villasante, and C. Tyler-Smith. 1989. Construction of yeast artificial chromosome libraries with large inserts using fractionation by pulsed-field gel electrophoresis. *Nucleic Acids Res.* **17:** 3425–3433.
14. Southern, E.M., R. Anand, W.R.A. Brown, and D.S. Fletcher. 1987. A model for the separation of large DNA molecules by crossed field gel electrophoresis. *Nucleic Acids Res.* **15:** 5925–5943.
15. Burke, D.T., J.M. Rossi, J. Leung, D.S. Koos, and S.M. Tilghman. 1991. A mouse genomic library of yeast artificial chromosome clones. *Mamm. Genome* **1:** 65.
16. Rossi, J.M., D.T. Burke, J.C.M. Leung, D.S. Koos, H. Chen, and S.M. Tilghman. 1992. Genomic analysis using a yeast artificial chromosome library with mouse DNA inserts. *Proc. Natl. Acad. Sci.* **89:** 2456–2460.
17. Featherstone, T. and C. Huxley. 1993. Extrachromosomal maintenance and amplification of yeast artificial chromosome DNA in mouse cells. *Genomics* **17:** 267–278.
18. Shibuya, H., K. Irie, J. Ninomiya-Tsuji, M. Goebl, T. Taniguchi, and K. Matsumoto. 1992. New human gene encoding a positive modulator of HIV Tat-mediated transactivation. *Nature* **357:** 700–702.

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