

Evolution of the Vertebrate Y RNA Cluster

Axel Mosig^{a,b}, Meng Guofeng^a, Bärbel M. R. Stadler^b,
Peter F. Stadler^{c,d,e,f,g,*},

^a*Department of Combinatorics and Geometry (DCG),
MPG/CAS Partner Institute for Computational Biology (PICB),
Shanghai Institutes for Biological Sciences (SIBS) Campus, Shanghai, China*

^b*Max Planck Institute for Mathematics in the Sciences,
Inselstrasse 22, D-04103 Leipzig, Germany*

^c*Bioinformatics Group, Department of Computer Science, University of Leipzig,
Härtelstraße 16-18, D-04107 Leipzig, Germany*

^d*Interdisciplinary Center for Bioinformatics, University of Leipzig,
Härtelstraße 16-18, D-04107 Leipzig, Germany*

^e*Department of Theoretical Chemistry
University of Vienna, Währingerstraße 17, A-1090 Wien, Austria*

^f*RNomics Group, Fraunhofer Institut für Zelltherapie und Immunologie — IZI
Deutscher Platz 5e, D-04103 Leipzig, Germany*

^g*Santa Fe Institute,
1399 Hyde Park Rd., Santa Fe, NM 87501, USA*

Abstract

Relatively little is known about the evolutionary histories of most classes of non-protein coding RNAs. Here we consider Y RNAs, a relatively rarely studied group of related pol-III transcripts. A single cluster of functional genes is preserved throughout tetrapod evolution, which however exhibits clade-specific tandem duplications, gene-losses, and rearrangements.

Key words: Y RNA, non-coding RNAs, evolution, gene duplications

1 Introduction

Y RNAs were discovered as the RNA component of the Ro RNP particle a quarter of a century ago [12]. Y RNAs form a small family of short polymerase III transcripts [18, 14, 4, 13] that are located in close proximity in the genomes of human, mouse and xenopus. The molecules exhibit a characteristic

secondary structure that has been extensively studied in the past both computationally [5] and by means of chemical probing [26]. The conserved structural features are required for binding the *Ro60* and *La* proteins [24, 8] and nuclear export [22]. A recent study [3] demonstrates a direct role of Y RNAs for DNA replication.

Although the conservation of Y RNAs among mammals has long been recognized [21, 6], there is to-date no systematic study of their molecular evolution. In addition to vertebrates, Y RNAs so far have been reported only in the nematode *C. elegans* [28] and the prokaryote *Deinococcus radiodurans* [2]. In primates, Y RNAs are the founders of a family of about 1000 pseudogenes that constitute a class of L1-dependent non-autonomous retroelements [19]. In contrast, in almost all other species (with the notable exception of the guinea pig *Cavia procellus*) there are only a few Y-RNA derived pseudogenes.

In this short contribution we describe a comprehensive analysis of Y RNA evolution in vertebrates based on the currently available genomic data including un-assembled shot-gun traces.

2 Materials and Methods

The genomes in the ENSEMBL database (release 41) were searched with `blast` and `ssaha2` for orthologs of the human and xenopus Y RNAs compiled in the Rfam database. The resulting sequences were then used to search the vertebrate sequences contained in the NCBI trace archive for additional orthologs. The sequences were then aligned using both `clustalw` [27] and `dialign` [15], resulting in four unambiguously discernible paralog groups corresponding to the four classes of human Y-RNA genes (Y1, Y3, Y4, Y5) for all amniote species. Of the four *Xenopus* sequences, xY3 and xY4 can be unambiguously assigned to the Y3 and Y4 paralog groups, respectively. For fully or partially assembled genomes we furthermore recorded the genomic locations.

These alignments were then used to derive patterns for `fragrep` [16], a tool that searches genomic DNA for short highly conserved patterns with intervening sequences of variable length. We used here an improved version that matches position-specific weight matrices (PWMs) rather than exact sequence patterns against the genomic DNA. We used `fragrep` to search the genomes of teleost fishes as well as non-vertebrate deuterostomes for Y RNA homologs. Alignments of the members of the individual paralog groups are provided as electronic supplement.¹

¹ <http://www.bioinf.uni-leipzig.de/Publications/SUPPLEMENTS/07-004/>

vergent, probably pseudogenized, copy of Y5 has been found. In contrast, all four Y RNAs appear to be intact in *Dipodomys ordii*, the fourth representative of the Sciurognathi. In all the three Cetartiodactyla (cow, pig, and bottlenose dolphin), no trace of a Y5 gene was found.

The afrotherian genomes (elephant, tenrec, and procavia) contain highly divergent Y1 sequences, which, judging from the increase rate of evolution, is likely to be a pseudogene, Fig. 1.

An interesting feature of the eutherian Y RNA cluster is the reverse orientation of the Y1 gene, Fig. 2. This character is not shared by both Platypus and Chicken. In these two species, as well as in the frog, all Y RNAs are co-linear. In contrast, the opossum genome exhibits a different rearrangement. This suggests that platypus represents the ancestral state in amniota.

In xenopus, we find that xYa, xY4 and Y4 are linked with EZH-2 and CUL-1 as in amniotes. The Y3 gene is located on the same scaffold. It is however, separated by 270kb and 4 protein-coding genes.

In teleosts, only a single Y-RNA homolog is detectable [16]. The same loci have also been identified in ENSEMBL release 42 using *infernal* [17] in fugu, tetraodon, medaka, and zebrafish. In zebrafish, medaka, and stickleback, the Y RNA gene is located on the same chromosome as homologs of CUL-1, EZH2, and PDIA4, although not in close proximity to these genes.

A single Y RNA candidate was identified in the genome of the lancelet *Branchiostoma floridae*. Fig. 3 shows that the sequence has plausible homology with the human Y-RNAs, in particular at the ends, i.e., in the characteristic stem region [5, 26]. Using *RNAfold*, one finds that the sequence can form a stem-loop structure as a suboptimal structure just about 2kcal above a V-shaped groundstate structure. A search across the genomes of the urochordates *Ciona intestinalis* and *Ciona savignyi* and of the echinoderm *Strongylocentrotus purpuratus* was not successful.

The phylogenetic analysis of the Y RNA sequences is complicated by the short sequence length (between 74 and 114bp) and by the divergence of the paralog groups outside the very well-conserved stem-region. The pattern of in/dels strongly suggests that the tetrapod Y1, Y3, Y4, and Y5 groups are monophyletic. The xY5 and xYa sequences are clearly recent paralogs and are both consistently placed within the Y5 group by both visual inspection of alignments and the phylogenetic analysis in Fig. 4. The bootstrap support for monophyletic Y1, Y3, Y4, and Y5 groups increases if the lancelet candidate is excluded from the analysis (data not shown). The support for a monophyletic clade of teleost Y RNA increases to more than 30% when the partial trout sequence is removed from the dataset.

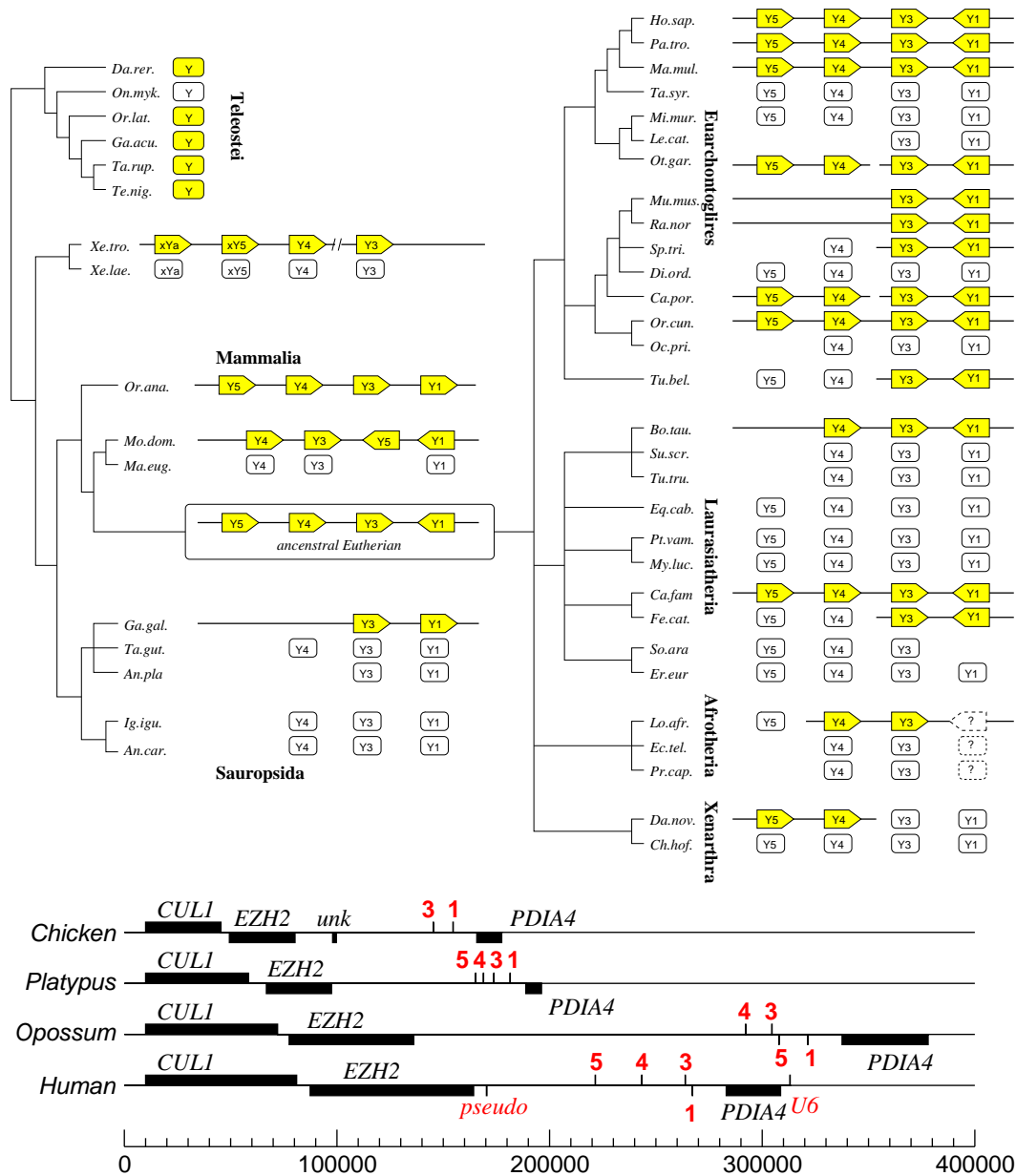


Fig. 2. Evolution of the vertebrate Y locus. (Top) schematic overview. With the exception of *Xenopus*, the functional Y RNA genes are located in a single cluster in all sufficiently assembled genomes (symbols with arrows on a line marking an uninterrupted piece of genomic DNA). For most species, only short scaffolds or shotgun traces are available (white symbols without direction). (Below) Comparison of Amniote Y RNA loci drawn to scale together with their flanking genes.

In addition to a classical NJ tree, Fig. 4a, we also computed a phylogenetic network to highlight the relatively large noise level in our data. Despite the rather high noise level, Fig. 4b also shows well-separated clades Y1, Y3, Y4, Y5, and teleost-Y. In order to assess the branching order of the major groups

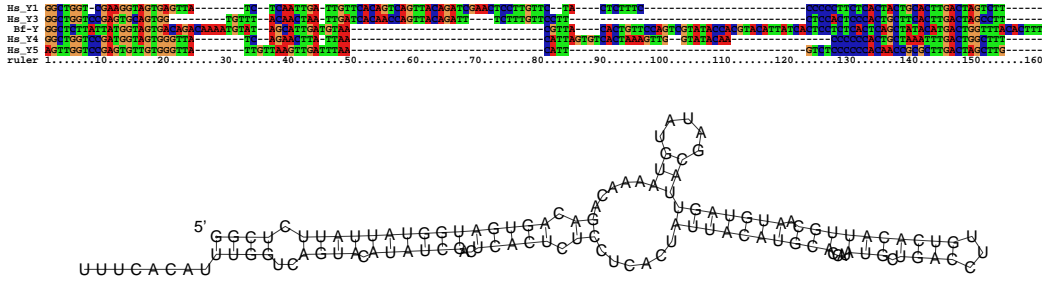


Fig. 3. A single candidate Y RNA sequence was found in the genome of *Branchiostoma floridae* using `fragrep`. The top panel shows an unedited `dialign` alignment of the lancet Y RNA with the four human Y RNA sequences. The panel below shows the predicted RNA secondary structure which was obtained using the most exterior base pair as a constraint. This suboptimal structure is about 2kcal/mol above the ground state.

we constructed individual `dialign` alignments containing one member of each clade as detailed in the Methods section. Fig. 4c displays the 7 most frequently supported splits. The three best supported ones define the strict consensus tree that places the teleost Y RNAs as sister group to a (Y1,Y3) subgroup of tetrapods. The grouping of teleost-Y with tetrapod-Y3 is in the NJ is very poorly supported; in the light of the split analysis, it is most likely just noise.

4 Discussion

Our analysis can be summarized by the following scenario, which is consistent with and at least weakly supported by all data. Starting with a single ancestral Y RNA a first tandem duplication produced pre-(Y4,Y5) and pre-(Y1,Y3) in this order on the genomic DNA. Presumably this event happened before the split of actinopterygians and sarcopterygians. The pre-(Y4,Y5) appears to have been lost in extant teleosts. Note that this first step is the one with the least direct support.

In the tetrapod ancestor, pre-(Y4,Y5) and pre-(Y1,Y3) then gave rise to the ancestral arrangement Y5→Y4→Y3→Y1. An additional duplication of Y5 occurred in the xenopus lineage. Loss of Y RNAs is an abundant phenomenon: Y1 was lost in xenopus, Y4 and Y5 was repeatedly lost in various amniote lineages (see Fig. 2). In mammals we furthermore observe different rearrangements of the Y RNA locus in both metatheria and in the eutherian ancestor.

As is the case with other non-coding RNAs, in particular pol-III transcripts, Y RNAs give rise to extensive families of (retro)pseudogenes [19]. In contrast to other classes, however, the locus of the functional originals has remained surprisingly stable. Despite frequent retrotransposition events and the fact

that the Y-RNA genes are independent pol-III transcripts, the functional Y RNA genes have remained within single uninterrupted clusters (with the exception of the situation in *Xenopus tropicalis*) throughout the evolution of tetrapods. In this respect, Y RNAs appear to behave differently compared other non-coding RNAs whose evolution has been studied in some details, including microRNAs [25, 9, 23, 20] and snoRNAs [1, 29, 30]. In microRNAs, preservation of genomic clustering is easily explained by the fact that the clustered miRNAs are processed from a single polycistronic primary transcript, see e.g. [11]. Other ncRNAs, in particular tRNAs, snoRNAs, and snRNAs behave more like mobile genetic elements [1, 29].

Acknowledgments

Discussions with Michael M. Müller is gratefully acknowledged. This work has been funded, in part, by the German DFG Bioinformatics Initiative BIZ-6/1-2.

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