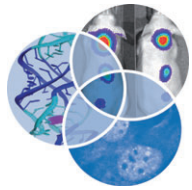
Advanced Review



Transcending the prediction paradigm: novel applications of SHAPE to RNA function and evolution

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Selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) provides information on RNA structure at single-nucleotide resolution. It is most often used in conjunction with RNA secondary structure prediction algorithms as a probabilistic or thermodynamic restraint. With the recent advent of ultra-high-throughput approaches for collecting SHAPE data, the applications of this technology are extending beyond structure prediction. In this review, we discuss recent applications of SHAPE data in the transcriptomic context and how this new experimental paradigm is changing our understanding of these experiments and RNA folding in general. SHAPE experiments probe both the secondary and tertiary structure of an RNA, suggesting that model-free approaches for within and comparative RNA structure analysis can provide significant structural insight without the need for a full structural model. New methods incorporating SHAPE at different nucleotide resolutions are required to parse these transcriptomic data sets to transcend secondary structure modeling with global structural metrics. These 'multiscale' approaches provide deeper insights into RNA global structure, evolution, and function in the cell.

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INTRODUCTION

As more than a mere messenger of information from DNA to protein, RNA molecules adopt structures that have functional biological roles.[1–6] Traditionally, secondary structure predictions of RNA molecules from a single sequence minimize a free energy function to obtain a structural model of intramolecular base pairing,[7–10] or sample suboptimal structures from the partition function.[11,12] Evolutionary covariation, where two nucleotides vary in sequence across related RNAs while preserving base pairing ability, is particularly powerful for predicting structures, especially for bacterial and archaeal RNAs or for highly conserved RNA structures.[13–15] Structure prediction algorithms, however, only predict about half of all base pairs that occur in an RNA,[16,17] and some alignments may not have enough information for covariation analysis.[18] For these reasons, an experiment capable of rapidly probing RNA structure is particularly appealing.

Multiple quantitative methods to determine RNA structure experimentally are now available, such as selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE),[19] quantitative dimethyl

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sulfate (DMS) modification,[20] and parallel analysis of RNA structure (PARS).[5,21] Each of these methods provides information on the conformational flexibility of nucleotides in an RNA, either through chemical modification (SHAPE and DMS) or through enzymatic probing (PARS). These quantitative probing techniques present a new paradigm in understanding RNA structure on a transcript-wide or greater scale.

The most common use for SHAPE data is as a restraint (also referred to as a soft constraint) in secondary structure prediction algorithms.[18,22–24] The incorporation of SHAPE data into structure prediction algorithms refines the probable structure space of an RNA molecule and greatly improves predictions to approximately 90% accuracy.[22,25] Recently, SHAPE data collected in an ultra-high-throughput manner is increasingly used in a model-free approach as an additional feature for evolutionary analysis. In this review, we discuss these diverse approaches to applying SHAPE data to understand RNA structure. As next-generation sequencing now allows for the rapid quantification of structure probing,[5,25–27] the future of SHAPE will involve signal processing techniques to understand a transcript's structure on multiple scales. The speed at which these technologies now provide us with structure information will allow for efficient and accurate analysis of comparative RNA structure.

FIRST-LEVEL APPROACHES TO INTERPRETING SHAPE EXPERIMENTS

SHAPE uses the reactivity of the 2′-OH of an RNA molecule to understand the structure of that RNA.[19,28–31] An electrophile, typically 1M7 or NMIA, covalently bonds with the 2′-O to form an adduct; this reaction occurs preferentially at conformationally flexible, or unpaired, nucleotides.[28,29] The signal is then 'read' by reverse transcription. In most protocols, modified nucleotides block the reverse transcriptase causing it to fall off the transcript. Nucleotides that are more reactive will generate more stops, which, as the experiments are traditionally performed with single-hit kinetics, indicates the relative frequency of adduct formation.[30] The relative rates of adduct formation are then normalized to find the SHAPE profile for that RNA, providing information on the reactivity of each nucleotide.[19,32]

In general, positions in a SHAPE profile with high reactivities are more likely to be unpaired, and positions with low reactivities are more likely to be paired.[22] Because the SHAPE reagent can react at any nucleobase, the SHAPE profile provides high-resolution structural information.[33] Indeed, differences in SHAPE profiles of two sequence variants indicate that an RNA is a riboSNitch, where a single nucleotide variant changes the structure of the RNA.[34–36] Thus, the SHAPE profile alone encodes information on an RNA's structure.

Recently whole-transcriptome probing methods use the power of next-generation sequencing with SHAPE structural profiling for an ultra-high-throughput way of probing RNA structure, such as the techniques *in vivo* click SHAPE (icSHAPE)[37,38] and SHAPE-Seq,[26,39,40] both of which utilize reverse transcription stops. Of particular interest, SHAPE with mutational profiling (SHAPE-MaP) uses modified reverse transcription conditions to induce mutations at positions with 2′ adducts, rather than causing a reverse transcription stop.[25] This new technique thus allows for high-resolution quantification of RNA structure for whole transcripts at every nucleotide position, without concerns about signal decay or adaptor ligation bias.[41–43]

What Is SHAPE Really Saying?

SHAPE is a powerful technique for probing the structure of an RNA but the results of a SHAPE experiment are not directly interpretable. Although SHAPE reactivities generally correspond to pairing state, the relationship between SHAPE and frequency of base pairing is not linear. Both Cordero et al.[44] and Sükösd et al.[45] found that the SHAPE reactivities of paired and unpaired nucleotides fall into two different probability distributions, although the two studies found different distributions of SHAPE reactivities for unpaired nucleotides (Figure 1(a)). While high (over 1.0) SHAPE reactivities generally only occur at unpaired nucleotides, lower SHAPE reactivities frequently correspond to both paired and unpaired nucleotides. Other structural factors such as base stacking may result in an unpaired nucleotide having a low SHAPE reactivity.[46] Consequently, the SHAPE reactivity alone is not always predictive of whether a base is paired or unpaired.

The distinction that SHAPE provides between paired and unpaired nucleotides is different between the data sets by Cordero et al.[44] (Figure 1(a); left) and Deigan et al.[22] (shown in Sükösd et al., Figure 1(a); right). The SHAPE data from Deigan et al. has a much larger difference between its paired and unpaired distributions. Thus, the strength of the SHAPE signal depends on the RNA and the experimental conditions, and is not merely a direct measurement of structure.[42,47] These factors are an important and often

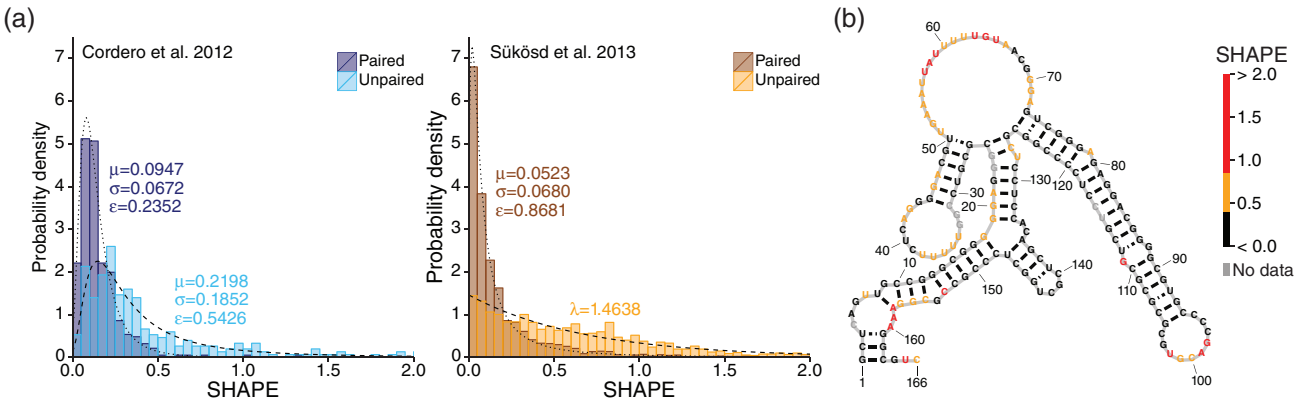

FIGURE 1 | SHAPE reactivities distinguish between paired and unpaired nucleotides. (a) Distributions of SHAPE reactivities for paired and unpaired nucleotides recreated from Cordero et al.[44] (left) and Sükösd et al.[45] (right; data from Deigan et al.[22]). SHAPE reactivities for paired nucleotides follow a generalized extreme value distribution in both data sets. SHAPE reactivities for unpaired nucleotides follow a generalized extreme value distribution in Cordero et al. and an exponential distribution in Sükösd et al. In both data sets, the distributions for paired and unpaired nucleotides differ, signifying that SHAPE reactivities are drawn from multiple probability distributions. (b) Example of RNA structure (the RB1 5′ UTR from Kutchko et al.[18]) overlaid with SHAPE data. Red: high SHAPE; orange: medium SHAPE; black: low SHAPE; gray: no data. Most paired positions have low SHAPE and many unpaired positions have high SHAPE, but SHAPE does not completely distinguish between paired and unpaired nucleotides, in part because this RNA forms multiple conformations.

underappreciated aspect of the experiment. The SHAPE reactivity profile depends on the overall fold of the molecule being probed. As such, experimental conditions can significantly alter the profile, and any comparative analysis of SHAPE reactivity should carefully consider experimental conditions.

SHAPE and Structure Prediction

Besides reporting a likelihood of base pairing for any given nucleotide, SHAPE data also refine secondary structure predictions of an RNA as an energetic restraint. This method was first introduced by Deigan et al.,[22] where an additional energy term to the nearest neighbor rules incorporates SHAPE reactivity as an additional pseudo-free energy term.[48] A high SHAPE reactivity makes that nucleotide less likely to base pair, while a low SHAPE reactivity makes base pairing more favorable. The SHAPE reactivities thus refine the structural space to find a structure or structures compatible with the SHAPE data (Figure 1(b)). As RNAs often adopt multiple structural conformations and as SHAPE is a measurement over the entire structural ensemble, the SHAPE data may not match a single structure but instead represent the average reactivity over the ensemble.

Still, projecting SHAPE reactivities onto a single structure, as is the case in Figure 1(b), is the most common approach for visualizing SHAPE data. Although one structure may appear to agree with the data (as in Figure 1(b)), there are often many other structures in the suboptimal ensemble that appear to agree with the data just as well. In fact, in the case of the *RB1 5′* UTR illustrated in Figure 1(b), three alternative conformations are all compatible with the data.[18] Nonetheless, there is value in visualizing structural models with SHAPE data projected as in Figure 1(b), and these approaches to interpreting SHAPE data will likely remain popular.

The most fitting way of incorporating SHAPE data into structure prediction is a subject of recent debate and thoroughly reviewed in Eddy 2014.[49] These methods all attempt to use SHAPE data to constrain structure prediction of that RNA, based on the likelihood of each nucleotide being paired. These methods treat SHAPE measurements in a variety of ways: as a free energy term,[22] as a prior probability,[50] or as a likelihood of base pairing.[24,51] While each method of incorporating SHAPE into prediction greatly improves the accuracy of the structure predictions, they all perform similarly to each other, with accuracies up to 90–95%.[22,24,25,44]

When given a SHAPE reactivity, it is impossible to know whether that base is paired or unpaired, especially at lower reactivities. Using structural probing as a restraint in structure prediction provides more information on the structure, with approximately the same accuracy over a wide range of parameters regardless of the method used.[22,24,25,44,50,51] The next challenge in SHAPE processing is not optimizing these parameters further, but instead evaluating broader structural characteristics that the SHAPE signal can inform us of. Given that the use of SHAPE in structure prediction was very extensively reviewed

recently by Eddy 2014,[49] we instead decided to focus on recent developments in the analysis of SHAPE signal that attempt to transcend the interpretation of the data as merely informative of a single secondary structure.

RECENT APPLICATIONS OF THE SHAPE SIGNAL

While SHAPE is certainly useful in refining RNA secondary structure prediction, it also provides additional information when analyzed at different scales, or when used in conjunction with other metrics such as evolutionary data. Here, we will discuss recent extensions of SHAPE reactivities beyond structure prediction.

Zooming Out: Regional View of SHAPE Reactivities

At a single-nucleotide resolution, the traditional conceptualization of SHAPE data is that each reactivity provides a likelihood of that base being paired or unpaired (Figure 2(a)). However, the proportions of unreactive (low-SHAPE) and reactive (high-SHAPE) nucleotides are not consistent across larger RNAs because while certain regions within a transcript fold into a well-defined structure, other regions may adopt multiple distinct structures with similar free energies.

It is important to note that a majority of transcribed RNAs are long, ranging up to several kilobases.[54,55] The longest transcripts are often RNA virus genomes, such as the well-studied HIV genome. To quantify these different SHAPE patterns, the median SHAPE reactivity over windows ranging from 50 to 75 nucleotides is a novel way to identify and visualize structured regions in a transcript.[25,52] Although SHAPE reactivities are at single-nucleotide resolution, averaging reactivities over many nucleotides reveals structured and unstructured regions of an RNA. Such 'multiscale' level analysis of these very large transcripts (often greater than 10 kb) provides a different picture of RNA structure than SHAPE at single-nucleotide resolution (Box 1). Of course, differences in the window size will change the scale at which we are understanding an RNA's structure, and the choice of window size to date has remained largely empirical. There are significant opportunities for algorithm development using SHAPE data at different scales.

Pollom et al.[52] compares the windowed SHAPE reactivities of both simian immunodeficiency virus (SIV) and HIV-1 (Figure 2(b)). Regions where both viruses have low median SHAPE correspond to known RNA structural elements such as the 5′ UTR, the gag-pro-pol frameshift, and the Rev response element. Other unannotated regions where both viruses have low median SHAPE reactivities are good targets for identifying new functional RNA elements. This windowed picture of SHAPE reactivity provides structural information—identifying regions of the RNA genomes that have well-defined structures—that is difficult to see at single-nucleotide resolution. Median SHAPE helps characterize structure across the transcript, demonstrating that SHAPE profiles have relevance beyond single nucleotide measurements and structure prediction algorithms. Future methods of processing these large SHAPE profiles, using this multiscale approach, may uncover more higher-order RNA features. This approach also identifies unstructured regions, which may turn out to be just as functional as structure.[18]

SHAPE Supplements Weak Evolutionary Signals

The traditional method of identifying conserved RNA structures is through mutual information or covariation of nucleotide positions.[13,14,56] Although covariance is extremely useful for highly conserved RNA structures such as the RNA component of the ribosome,[15,57] the covariance signal may be weak or nonexistent in other eukaryotic RNAs, making it difficult to identify conserved RNA structures in these organisms.[18] SHAPE represents a new method to help strengthen the evolutionary signal by finding support for conserved RNA structures independent of structure prediction.

Kutchko et al.[18] looked at the structure of the 5′ UTR of *RB1*, the transcript that codes for the tumor suppressor Retinoblastoma protein, in three different species. Qualitatively, SHAPE data for the three different UTRs have regions of striking similarity (Figure 2(c)). As in reference by Pollom et al.,[52] SHAPE data—even in the absence of structure prediction—can indicate structural similarity between different sequences. In addition, SHAPE data can provide insight into observed evolutionary patterns. Watts et al.[23] used SHAPE to find that hypervariable regions of the HIV-1 genome are highly unstructured and insulate low-SHAPE, highly structured helices. When used in this manner, SHAPE adds a layer of context to evolutionary patterns (Box 2).

SHAPE-Directed Sequence Alignments Identify Conserved RNA Structures

To extend beyond qualitative analysis, the Weeks lab recently published two papers using SHAPE data as

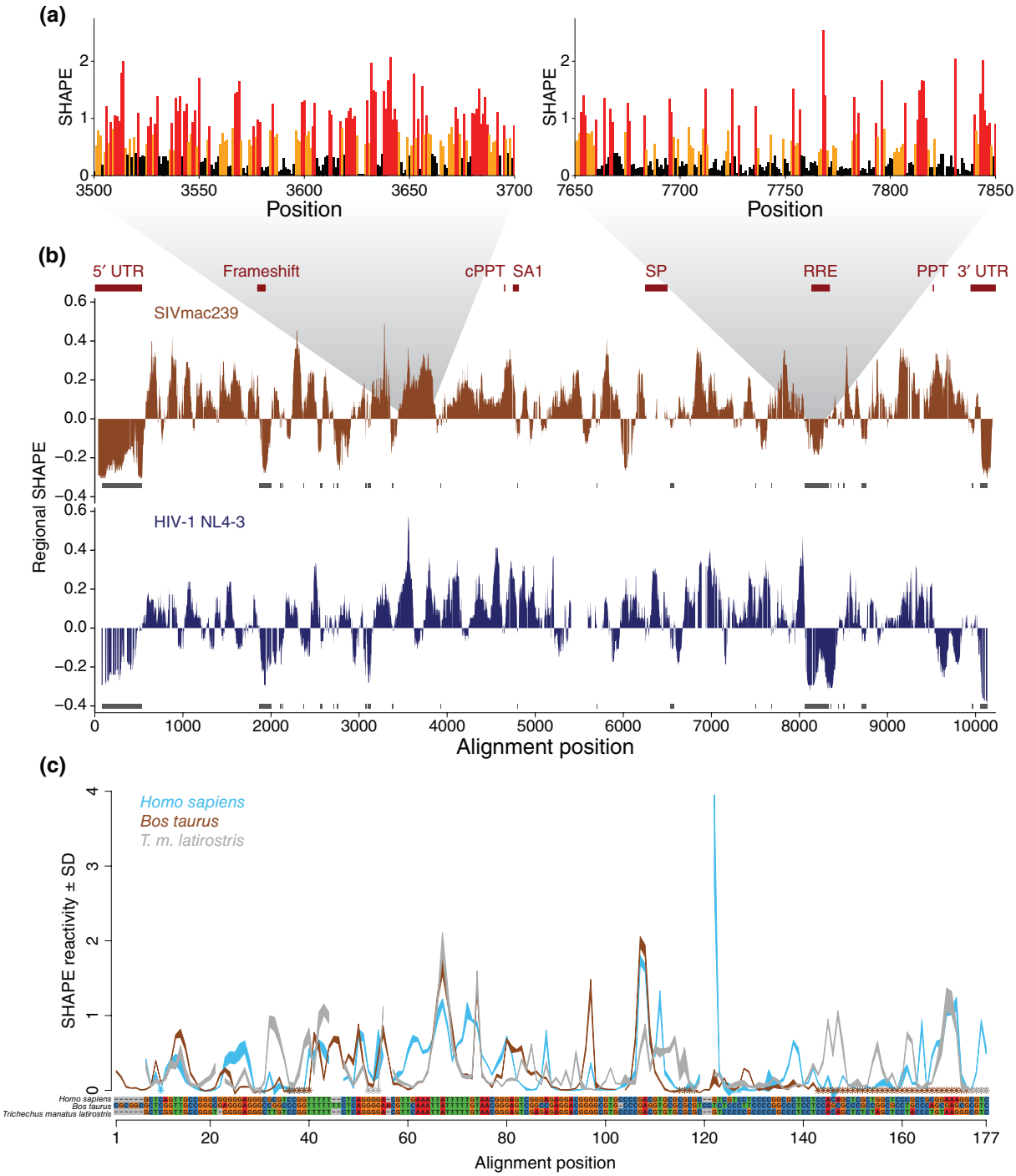

FIGURE 2 | SHAPE data for related RNAs follow similar but not identical patterns. (a) Examples of SHAPE data from high-SHAPE regions (left) and low-SHAPE regions (right) from the SIVmac239 SHAPE data from Pollom et al.[52] High SHAPE nucleotides are indicated in red, medium in orange, and low in black. Although both profiles have low- and high-SHAPE nucleotides, the frequencies of each are distinct between the two regions. (b) SHAPE data for SIVmac239 (top) and HIV-1 (bottom) aligned genomes. SHAPE data are from Pollom et al., annotations are from Pollom et al. and the Los Alamos HIV database (http://www.hiv.lanl.gov/), and the sequences were aligned using MAFFT.[53] Regional SHAPE represents the windowed median SHAPE over a 75-nt window, with respect to the global median SHAPE value for each transcript. Values above the line are regionally unstructured, and below the line are regionally structured. Alignment regions where both viruses are regionally structured are annotated in gray. These regions correspond to known structural elements of the SIVmac virus (above, red). (c) SHAPE reactivities aligned by sequence for the *RB1* 5′ UTR in human (blue), cow (brown), and manatee (gray) from Kutchko et al.[18] The SHAPE profiles for each species are similar across most of the UTR. (Reprinted with permission from Ref 18. Copyright 2015 Kutchko et al.; Published by Cold Spring Harbor Laboratory Press for the RNA Society).

a feature for sequence alignment.[58,59] Their algorithm aligns two SHAPE profiles to minimize the difference in SHAPE reactivity at each position, optionally including sequence as an alignment parameter (Figure 3(a)). The difference in SHAPE reactivity between two sequence positions becomes a function applied to the Gotoh alignment algorithm[60] (Figure 3(b)). This novel method uses SHAPE reactivity as an additional feature for understanding evolutionary conservation.

As a proof of concept, they used the method to align ribosomal RNA sequences from *Escherichia coli*, *Clostridium difficile*, and *Haloferax volcanii* with only SHAPE data, not taking sequence into account[58] (Figure 3(c)). It should be noted that aligning these distantly related rRNAs based on sequence alone is quite challenging.[58] The rRNAs aligned with SHAPE data alone produce an alignment with similar accuracy to the sequence-only alignment, and using both sequence and SHAPE data reproduce the gold standard, manually curated alignment, suggesting that SHAPE data captures evolutionary patterns in RNA structure.

To extend this method further, they aligned three lentivirus genomes (HIV-1, SIVcpz, and SIVmac) using sequence and SHAPE together[59] (Figure 3(d)). Some regions in viral genomes have very robust structures, although unlike rRNA, viral RNAs also have many unstructured or less-structured regions. The application of SHAPE-directed sequence alignments to viral RNAs is thus an important test of this new alignment method. From the aligned SHAPE data, they used a linear regression model to find regions where the SHAPE profiles are correlated between the three viruses (Figure 3(e)). Known RNA functional elements have significant correlations between the SHAPE profiles (Figure 3(e); green), indicating that SHAPE correlation is a signal for structural homology.

SHAPE-directed sequence alignments are therefore applicable to more divergent RNAs, but most useful for conserved RNA elements where the SHAPE profiles are similar. Systematically using SHAPE as an alignment parameter is a novel approach to identifying conservation of RNA structure, and future extensions of this technique may involve quantifying structural divergence and similarity using SHAPE data.

SHAPE Facilitates Discovery of the Conservation of Multiple RNA Structures

The SHAPE signal does not measure the conformation of a single molecule but instead represents the average reactivities over all copies of that RNA transcript. Indeed the reaction occurs in bulk, and as such the reactivity at any given nucleotide is an ensemble average. As many RNA structures, including riboswitches, adopt multiple conformations,[2,61] it is critical to consider the entire structural ensemble of a transcript when interpreting the SHAPE profile. We can therefore compute the SHAPE-directed partition function, which models the entire structural ensemble informed by SHAPE data[11,22] and allows us to identify the presence of multiple structural conformations.

As comparative structure analysis now incorporates SHAPE data as additional information, the next step in this direction is using SHAPE to compare multiple structural conformations of an RNA. Our laboratory recently showed that multiple conformations of a purine riboswitch are conserved in sequence[62] (Figure 4(a)). However, the covariation signal is much weaker in eukaryotic RNAs than bacterial or archaeal. SHAPE data thus provide us with more information, helping us identify the conservation of multiple structures.

To incorporate SHAPE into conservation analysis, we used SHAPE to probe the 5′ UTR of the human RB1 transcript.[18] As SHAPE data greatly improve the accuracy of RNA secondary structure prediction[22,25] we can be confident in our subsequent computational analysis. Using SHAPE-directed Boltzmann sampling,[10,22,63] we found that the 5′ UTR adopts three distinct conformations (Figure 4(b)). We applied the same analysis to the homologous UTRs in *Bos taurus* and *Trichechus manatus latirostris* and found that they also adopt multiple, similar conformations to the UTR in humans (Figure 4(c)). Here, in conjunction with structure prediction, SHAPE helps uncover the conservation of multiple structures in an RNA. As whole-transcriptome probing technologies improve, we anticipate further discovery of this type of structural conservation and we encourage others investigating RNA structure to also investigate the conservation of multiple conformations as an important functional consideration.

THE FUTURE OF SHAPE IN THE NEXT-GENERATION SEQUENCING ERA

The recent developments of high-throughput structure probing technologies such as SHAPE-Seq,[39] icSHAPE,[31] and SHAPE-MaP[25] utilize next-generation sequencing to quickly obtain the SHAPE profile of one or more RNAs, making SHAPE now a next-generation probing technology. With the advent of next-generation sequencing, probing experiments

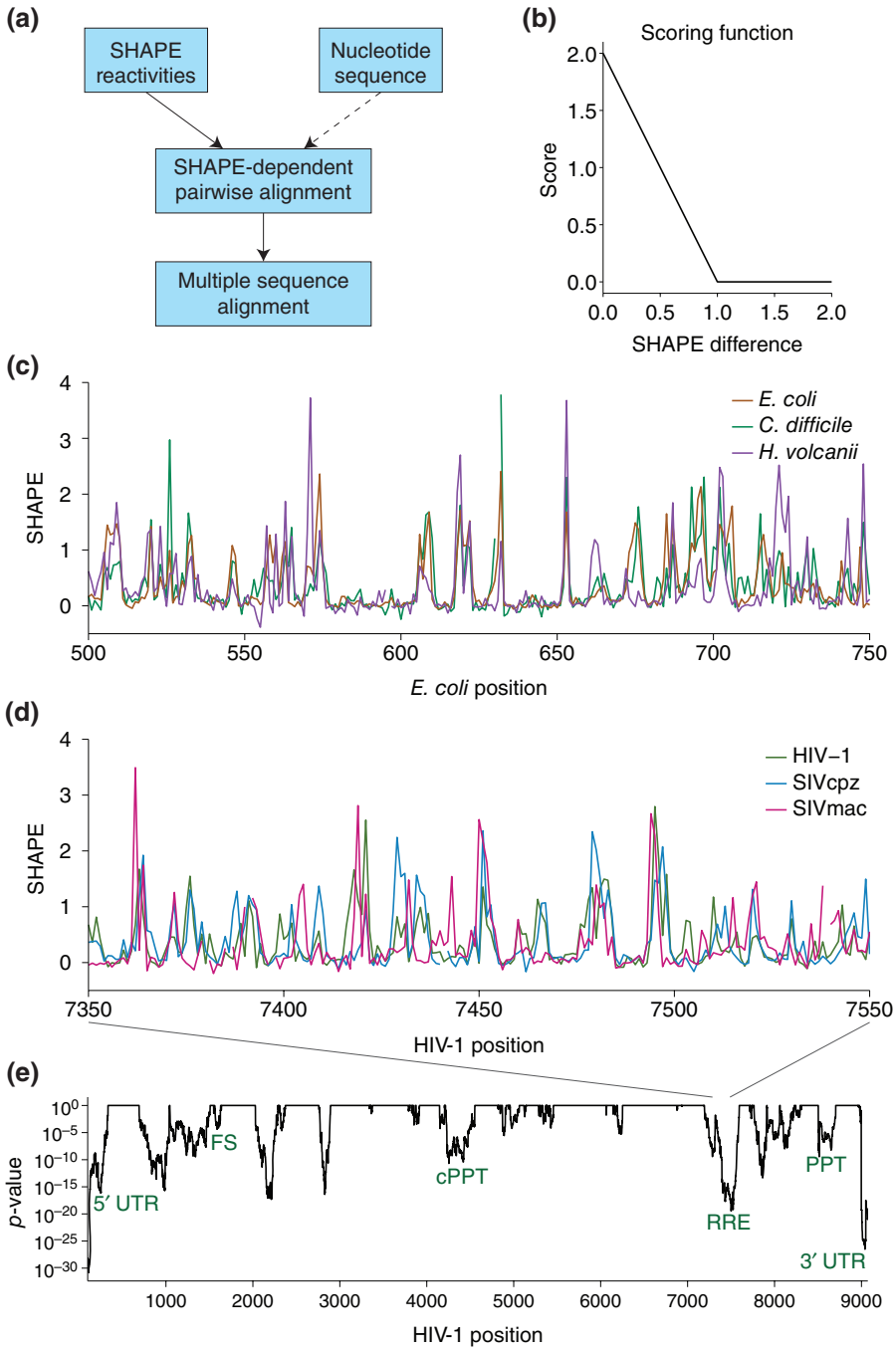

FIGURE 3 | SHAPE data of homologous RNAs can facilitate sequence alignments. (a) Schematic of SHAPE-directed sequence alignment used in Lavender et al. (1)[58] SHAPE reactivities, and optionally nucleotide identity, can be used as parameters in the Gotoh alignment algorithm for pairwise alignment. Pairwise alignments can then be combined to create a SHAPE-informed multiple sequence alignment. (b) Scoring function from Lavender et al. (1) used to align SHAPE reactivities for two sequences. More similar SHAPE reactivities have a higher score for alignment. If SHAPE reactivities differ by more than 1, the scoring function treats them as unrelated. (c) SHAPE-only alignment of a section from three 16S rRNA sequences. Data from Lavender et al. (1) The aligned SHAPE reactivities are very similar, reflecting structural homology. (d) SHAPE-directed multiple sequence alignment of three lentivirus sequences. The aligned SHAPE profiles of each virus have similar patterns. (e) Significance of the correlation of SHAPE between the three viruses, determined by linear regression. Lower *p*-values indicate more similarity in SHAPE profiles. The SHAPE profiles have regions of significant correlation across the alignment, particularly in regions known to have functional RNA structures. (Reprinted with permission from Ref 58 Copyright 2015 Lavender et al.; PLOS Computational Biology and Ref 59 Copyright 2015 Lavender et al.; PLoS Computational Biology).

are much faster and more efficient than in the past, and public databases such as the RNA Mapping Database[64] allow for large-scale comparisons of SHAPE data. Several studies recently applied next-generation sequencing to analyze RNA structure at the whole transcriptome level.[5,27,36,65,66] With this unprecedented amount of RNA structural probing data, the next steps in computational analysis of RNA structure will incorporate methods beyond structure prediction into read alignment, motif analysis, and signal processing.

In this new era of 'next-generation structural probing,' the SHAPE signal is now one of multiple available features to inform us about an RNA. SHAPE represents a model-free approach to RNA structure analysis and provides information at multiple levels, from single nucleotides to 50-nt windows to whole transcripts. This multiscale approach to SHAPE structural probing gives us a wealth of information on the structure of a transcript, and combined with other methods such as Shannon entropy[25,67,68] or mutual information,[14] provides us with great detail on the transcript's structure.

Using SHAPE at this whole-transcriptome, multiscale level will facilitate the application of signal processing methods to these SHAPE profiles of interest and uncover RNA structural features. The use of Fourier transforms to detect periodicity in (aggregated) SHAPE data is the first example of applying signal processing to SHAPE.[27,69] As new model-free methods are applied to SHAPE profiles, we will gain detailed information on RNA structure at a whole-

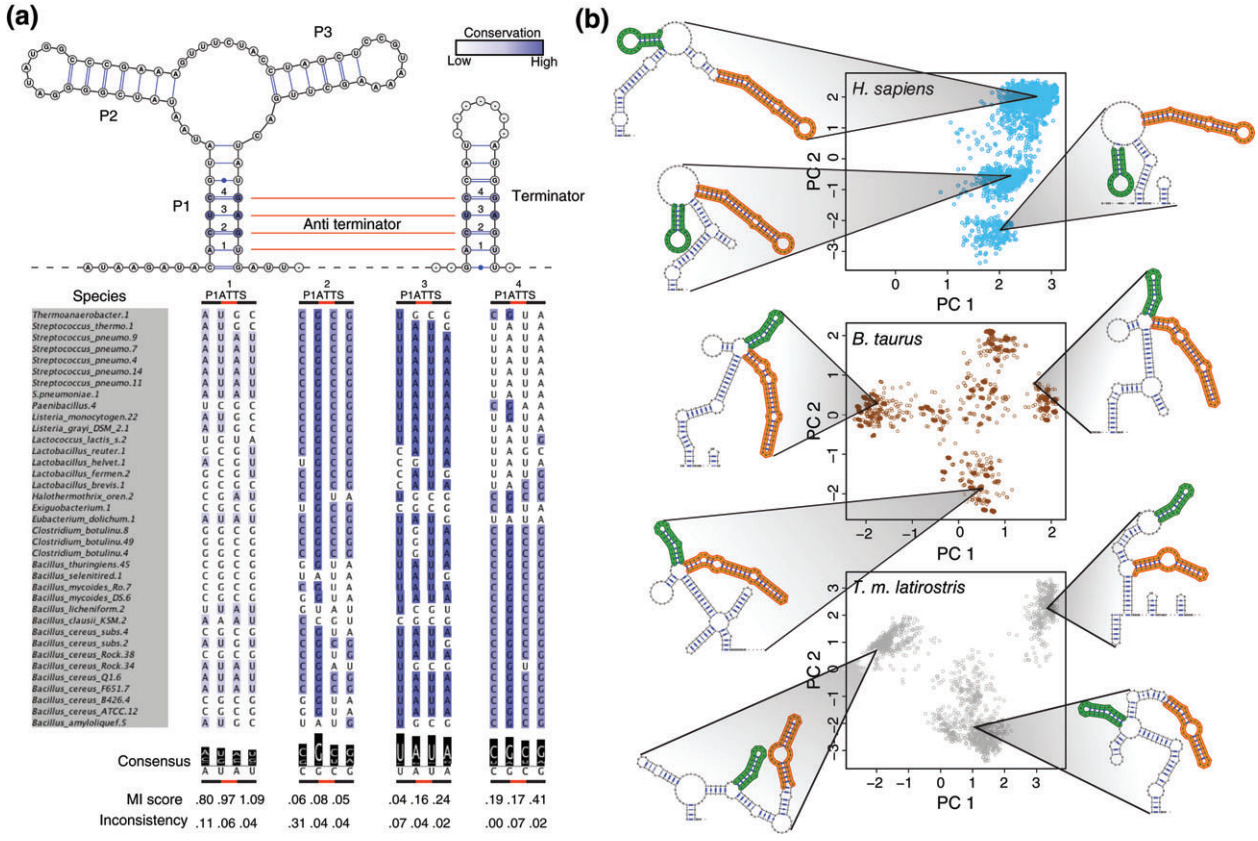

FIGURE 4 | SHAPE reactivities can help identify conservation of multiple RNA structures. (a) Figure from Ritz et al.[62] Multiple conformations of an RNA are evolutionarily conserved. Top: Purine riboswitch consensus structure with the anti-terminator pairs in red lines. Both the P1/terminator conformation and the anti-terminator conformation must be conserved. Bottom: Evolutionary analysis of bases involved in the P1, terminator, and anti-terminator stems. Blue indicates conservation of each base. Mutual information shows that the pairs involved in the anti-terminator stem preserve their ability to base pair, but also to pair with their partners in the P1 and terminator stems. (Reprinted with permission from Ref 62. Copyright 2013 Ritz et al.; PLOS Computational Biology) (b) The 5′ UTR of *RB1* forms multiple distinct structures in humans (blue), cow (brown), and manatee (gray), with SHAPE-directed Boltzmann sampled structures indicated by blue dots. Structures and arc diagrams on the side show representative structures from each conformation. Green and orange stems are conserved across all three organisms. Thus, SHAPE-directed structure prediction allows us to confidently identify the conservation of multiple RNA structures. (Reprinted with permission from Ref 18. Copyright 2015 Kutchko et al.; Cold Spring Harbor Laboratory Press).

BOX 1

NEW INTERPRETATIONS OF SHAPE FOR NEXT-GENERATION PROBING

Traditionally, researchers have used SHAPE to understand the structure of a single RNA. Recently, though, developments such as SHAPE-Seq, icSHAPE, and SHAPE-MaP now make SHAPE structural probing a next-generation technology. Now, SHAPE has more applications than just as a measure of whether a single nucleotide is paired or unpaired—it can indicate structuredness at multiple resolutions. SHAPE is now one of multiple metrics—including base pairing probability, Shannon entropy, and nucleotide content—for interpreting both local and broad structure of an RNA. The future of SHAPE as a genome-wide (for viruses) or transcriptome-wide (for bacteria and eukaryotes) measurement technique relies on signal processing of this large-scale data.

BOX 2

MEASURING RNA STRUCTURE CONSERVATION WITH SHAPE

As SHAPE is a model-free measure of RNA structure, correlations between SHAPE profiles can find common structures across homologous transcripts. Formal methods for this kind of analysis, such as using SHAPE as a sequence alignment parameter, will aid in finding conserved and functional RNA structures. The ability of technologies including SHAPE-Seq, icSHAPE, and SHAPE-MaP to generate large amounts of structural data very quickly presents a new opportunity to understand the evolution of RNA structure. New multiscale metrics, analyzing SHAPE data at multiple resolutions, can identify conserved structures, or structural ensembles, in RNAs throughout evolution.

transcriptome level and likely discover new levels of structural conservation.

In addition, these new high-throughput probing technologies allow us to generate SHAPE data for related RNAs very quickly, facilitating the comparison of SHAPE data and structural preservation between homologous sequences. As with HIV and SIV genomes,[59] correlations of SHAPE data will identify regions with conserved RNA structure. We live in exciting times for RNA structural biology as these next-generation structural probing techniques are being developed, refined, and applied to large data sets. The next few years will involve a huge expansion of information regarding the conservation of RNA structure.

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