Review

Adam E. Hall[a], Carly Turnbull[a] and Tamas Dalmay*

Y RNAs: recent developments

Abstract: Non-coding RNAs have emerged as key regulators in diverse cellular processes. Y RNAs are ~100-nucleotide-long non-coding RNAs that show high conservation in metazoans. Human Y RNAs are known to bind to the Ro60 and La proteins to form the Ro ribonucleoprotein complex. Their main biological function appears to be in mediating the initiation of chromosomal DNA replication, regulating the autoimmune protein Ro60, and generating smaller RNA fragments following cellular stress, although the precise molecular mechanisms underlying these functions remain elusive. Here, we aim to review the most recent literature on Y RNAs and gain insight into the function of these intriguing molecules.

Keywords: DNA replication; Ro60; small non-coding RNA; Y RNA.

[a]These authors contributed equally to this work.
***Corresponding author: Tamas Dalmay,** School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK, e-mail: T.Dalmay@uea.ac.uk
Adam E. Hall and Carly Turnbull: School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

Introduction

At the turn of the millennium, it became increasingly apparent that the role of RNA in cellular processes was much more significant than first thought (1). There are a vast number of distinct families that make up the 'RNA world' (2), having diverse roles such as gene silencing, intermediates during gene expression, non-protein-based enzymes, and splicing. Two characteristic features of these RNA families are their length and secondary structure.

Although RNA acts as an intermediary in the 'central dogma' of gene expression (the expression of genes from DNA to RNA, then to protein), the vast majority of cellular RNA is non-coding. Indeed, most of the genome in complex organisms is transcribed into non-coding RNA rather than protein coding transcripts (3). Only 1.5% of the human genome is protein coding, yet around 80% is transcribed into RNA (4), making non-coding RNA a dominant feature in the cellular landscape.

Y RNAs are one such family of non-coding RNAs. They were first identified in the early 1980s during investigations into autoimmune proteins and associated RNAs in systemic lupus erythematosus patients (5, 6). The four Y RNAs, Y1, Y3, Y4, and Y5 (Figure 1), are highly evolutionary conserved (7, 8), and have been shown to bind to a variety of proteins (9–14). The principal Y RNA-binding proteins appear to be Ro60 and La, which when bound to Y RNAs give rise to the Ro-RNP (Ro60 ribonucleoprotein) complex.

The Y RNA 'life cycle' begins with the Y RNA, and two key genes (*Ro60* and *La*) being transcribed in the nucleus. The *Ro60* and *La* transcripts are exported to the cytoplasm where they are translated into proteins, which are subsequently imported back into the nucleus. As RNA polymerase III-controlled transcription of the Y RNA is terminated, the newly synthesised transcript associates with La, followed by Ro60 (15). The entire Ro-RNP complex is then transported to the cytoplasm. However, some Ro-RNP complexes, such as the Y5 RNP, are retained in the nucleus (16).

Several excellent reviews are already available, which summarise the discovery and characterisation of Y RNAs and their associated proteins (17–19). Here, we shall review three more recent areas of Y RNA biology where there have been significant developments over the last 6 years. Research into the Y RNAs appears to have centred on, first, their involvement in DNA replication; second, the binding of Ro60 and associated proteins; and third, the biogenesis and function of Y RNA-derived small RNAs. Possible avenues of future research are also discussed.

The role of Y RNAs in chromosomal DNA replication

The propagation of life relies on the efficient and timely replication of the cell's genetic material. During cell division in all organisms, parental DNA gives rise to two identical daughter strands, each inheriting one original strand

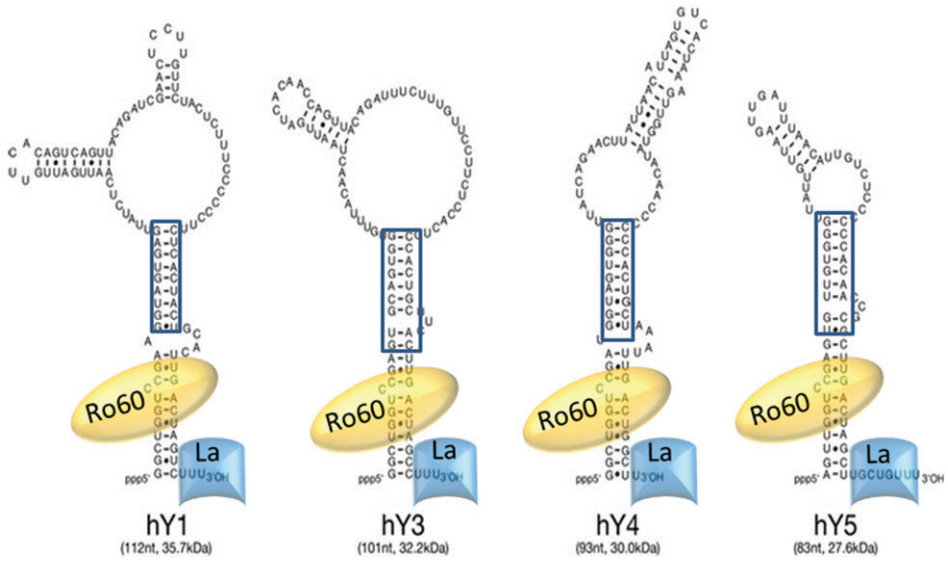


Figure 1 Y RNAs, associated binding proteins, and regions involved in DNA replication.
All Y RNAs have at least two main stems separated by a large internal pyrimidine-rich loop. The most conserved region between the Y RNAs is the stem at the 3′/5′ end. This is where the Ro60 protein binds, and involves the important cytosine bulge that is crucial for this interaction (13). Both enzymatic cleavage and chemical modification experiments have been used to determine these structures shown (9, 70). The region implicated in chromosomal DNA replication is boxed in blue (26). Nucleolin binds to the large internal loop structure (13, 14, 30); however, the binding sites of DNA replication associated proteins Cdt1 and Cdc6 are still to be established. Y RNAs have a 3′ poly (U) tail and a 5′ triphosphorylated guanine residue (pppG), except for Y5, which has pppA (12). Figure adapted from ref. (68).

from the parent in a semiconservative fashion. In eukaryotes, chromosomal DNA replication is tightly regulated and is closely linked to the cell cycle, occurring in S phase.

The current model of DNA replication is already well reviewed in the literature (20, 21); however, we will briefly summarise key molecular events here to facilitate the understanding of the role of Y RNAs in this process later in the review. Eukaryotic cells start DNA replication through the assembly of the multiprotein pre-replicative complex (pre-RC) in the G_1 phase of the cell cycle. This is initiated with the assembly of the six-subunit, origin recognition complex (ORC), which in turn recognises specific sites where DNA replication initiation occurs – called autonomously replicating sequences. The Cdc6 and Cdt1 proteins then interact with the ORC and facilitate the recruitment of the minichromosome maintenance proteins Mcm2–7. Together, these proteins form the pre-RC in G_1 phase nuclei. Activation of the pre-RC and the formation of DNA replication forks occurs during the transition to S phase and involves a second group of factors. These proteins include the two kinases Cdk2 and Cdc7. Other initiation factors are then recruited, including Cdc45, Mcm10, the GINS complex and replication protein A. Origin DNA is then unwound, and the three DNA polymerases (DNA pol α, DNA pol δ, and DNA pol ε) are recruited and commence replication.

Early studies that investigated the biochemistry of DNA replication used cell fusion experiments, such as those famously conducted by Rao and Johnson in 1970 (22). Using this technique, it was shown that S-phase cells contain factors that could induce G_1, but not G_2 phase nuclei, to prematurely trigger DNA replication. More recently, the development and optimisation of a human cell-free system to study DNA replication was established, which has resulted in further replication factors being uncovered (23, 24). In this cell-free system, isolated cell cycle-arrested template nuclei synchronised in late G_1 phase are prepared, which are licensed to replicate, but lack active DNA replication forks. Then, by adding cytosolic extracts from proliferating cells containing initiation factors or combinations of specific cellular fractions, novel DNA replication factors can be identified by seeing if arrested nuclei can then initiate DNA replication (24).

Using this cell-free system, Krude and colleagues (25) identified the non-coding Y RNAs as essential factors for chromosomal DNA replication. When the cytosolic extract from actively proliferating cells was fractionated by anion exchange chromatography, this gave rise to two essential fractions called QA and QB. QB was further separated into fractions ArFT and ArE. Fractions QA, ArFT, and ArE were sufficient to initiate DNA replication when added to template nuclei. Surprisingly, fraction ArE was found to be

composed mainly of nucleic acids rather than proteins, and it was from this fraction that Y RNAs were purified. The QA and ArFt fractions alone were not sufficient to significantly drive DNA replication in isolated nuclei from human somatic cells, with only 15% of nuclei replicating in the presence of these two fractions (25). However, when the purified Y RNAs were added along with these two protein fractions, the proportion of replicating nuclei increased in a dose-dependent manner to 50% (25). Furthermore, targeted depletion of Y RNAs from the cytosolic extract inhibited DNA replication. A degree of functional redundancy with regards to the Y RNAs was found in this system as addition of any of the four Y RNAs to the depleted extract was sufficient to reinstate DNA replication. This requirement for Y RNAs for DNA replication was also seen in mouse cell nuclei, where interestingly, human Y RNAs could also initiate mouse DNA replication, indicating that these factors are evolutionary conserved. Non-vertebrate Y RNAs could not reconstitute DNA replication in vertebrate systems (26).

Importantly, the structure of the Y RNAs was shown not to be the contributing factor that initiated DNA replication (25). Instead, a conserved double-stranded 9-base-pair motif sequence in the upper stem of the Y RNAs (Figure 1) identified in screens of hY1 mutants was sufficient to drive DNA replication in the presence of protein fractions QA and ArFt (26). This motif was found to have a consensus sequence of 5′-GUAGUGGG-3′ on the 5′ strand and 5′-CCCACUGCU-3′ on the 3′ strand. This motif was not found in non-vertebrate Y RNAs. The addition of this double-stranded sequence alone was sufficient to drive DNA replication without the requirement of the other protein binding domains of the Y RNA (again, in the presence of QA and ArFt). Interestingly, the same results were not observed with the addition of this sequence in DNA from.

One of the major functions of Y RNAs is the ability to bind the Ro60 protein, which has a role in RNA quality control (9, 10). Ro60 binds the lower stem of the Y RNA (Figure 1). Another protein called La binds the 3′ polyuridine tail (12, 13) and other proteins such as Nucleolin bind to the large internal loop structure of the Y RNA (13, 14). Langley and colleagues (27) showed that none of the associated Y RNA-binding proteins were required for DNA replication and that addition of any of these proteins did not affect the percentage of actively replicating nuclei in the cell-free system. It was found through real-time PCR that around 50% of cellular Y RNAs were not bound by Ro60/La/Nucleolin (27), and it was argued that it is the non-protein-bound portion of Y RNAs that have a role in DNA replication, whereas the Ro60/La/Nucleolin-bound Y RNAs carry out distinct functions such as scavenging for misfolded RNAs. Interestingly, other groups have shown through Northern blot-based studies that around 90% of cellular Y RNAs are bound by Ro60 (15, 28). This discrepancy in the literature remains to be clarified.

To investigate exactly how Y RNAs facilitate chromosomal DNA replication, Krude and colleagues (29) degraded Y1 and Y3 RNA at different time points of the cell cycle and monitored replication track extension rate (or the rate at which nascent DNA is polymerised). They found that Y RNAs are required for the establishment and initiation of DNA replication forks, but are not required for the elongation of actively replicating DNA. Additionally, the stability of the DNA replication fork was not affected by depleting the Y RNA population. The reduction in the amount of single-stranded nascent DNA by degrading Y3 RNA could be negated by the addition of Y1 RNA – further supporting the functional redundancy of Y RNAs in this process. In another study, using fluorescently labelled Y RNAs, it was shown that Y RNAs act in a 'catch and release' mechanism whereby Y RNAs associate with the unreplicated euchromatin in the late G_1 phase of the cell cycle, and are displaced once initiation has taken place (30). Around 20–70% of hYRNAs associate with nuclei in G_1 phase cells, whereas only 4–10% associate in G_2. It was further shown that hY1, hY3, and hY4 co-localise with each other on euchromatin sites, but not with hY5, which was enriched in nucleoli. Although it was previously shown that the upper stem region alone was sufficient to reconstitute DNA replication in template nuclei (26), it was demonstrated that the loop domain of the Y RNAs was required for targeting Y RNAs specifically to euchromatic sites. Y RNA mutants where only the upper stem maintained a wild-type sequence showed non-specific site binding across the nucleus, including heterochromatic regions (30).

Human Y RNAs appear to associate with DNA replication factors. This was demonstrated by conducting RNA pull-down assays where agarose beads are coupled to the 3′ termini of the Y RNA (30). All four Y RNAs interact with members of the origin recognition complex (ORC 2 and 3 interacted with hY1, 3, 4, and 5 and ORC 4 and 6 interacted with hY1, 3 and 5 only). Furthermore, Cdt1, Cdc6, and the DNA unwinding protein DUE-B all interacted with the Y RNAs. None of the proteins associated with DNA replication elongation such as Mcm2–7, CMG, and the DNA polymerases bound to the Y RNAs, supporting earlier work that these non-coding RNAs are specifically involved in the initiation stage of replication (29, 30).

Finally, the most recent work with regards to Y RNAs and chromosomal DNA replication showed that the Y RNAs only act as licensing factors after the midblastula

transition (MBT) stage of development (31) and not before. Using inhibitory morpholino nucleotides, it was demonstrated that Y RNAs are not needed for DNA replication in *Xenopus* and zebrafish embryos and egg extracts before MBT. After this stage, Y RNA-specific morpholino nucleotide-treated embryos died shortly before gastrulation. The lack of requirement for Y RNAs to play a role in DNA replication before MBT may facilitate the need to replicate the cell's DNA rapidly during early developmental stages. After this developmental stage, DNA replication takes significantly longer – such as in post-MBT cells.

Y RNAs and associated proteins

All human Y RNAs can bind to both the Ro60 and La proteins (6, 9, 12). La carries out a multitude of different functions within the cell, such as the stabilisation of RNA polymerase III transcripts and the processing of tRNAs, which has been reviewed elsewhere (19). There is mounting evidence that Ro60 is involved in RNA processing and quality control (10, 18, 32–36).

In 2005, the 'steric occlusion' model was put forward, suggesting that Y RNAs block the binding of variant or misfolded ncRNAs to Ro60; Y RNAs bind to the outer surface of the Ro60 protein, while ncRNAs interact with an overlapping region on the outer surface, as well as in the central cavity (36–38). In 2007, Hogg and Collins contested this model, hypothesising that Y RNAs actually recruit misfolded or variant ncRNAs to Ro-RNP complexes. This group went on to show that Y5 interacts with 5S ribosomal RNA (rRNA) through the ribosomal protein L5 and that Y5 prefers ectopically expressed variant 5S rRNA over endogenously expressed wild-type 5S rRNA (35). It is possible, and indeed the group themselves propose, that both of these models are true under different circumstances.

A third hypothesis is that Y RNAs modulate the interaction of Ro60 with ncRNAs by altering Ro60 localisation. Sim et al. (39) used chimeric proteins, produced by combining bacterial Ro60 domains with mouse Ro60 domains, to show that vertebrate Ro60 contains a nuclear localisation signal in helices 1–12 of the HEAT domain. This domain overlaps the region that interacts with Y RNAs. During UV or oxidative stress, the mouse Ro60 protein relocates to the nucleus (34, 39), while the bacterial Ro60 protein remains partially nuclear and partially cytoplasmic (39). Targeted knockdown of mouse Y3 RNA by siRNAs results in the relocation of Ro60 to the nucleus in the absence of stress. However, when mY1 is degraded after siRNA knockdown, the resulting fragments still interact with Ro60 and this complex remains cytoplasmic (39). Mutating the Y RNA-binding region of the mouse Ro60 protein also leads to nuclear accumulation in the absence of stress (39). This indicates that interacting mouse Y RNAs retain Ro60 in the cytoplasm. In human cells, the Y5 RNA is reported to be primarily nuclear (16). It would be interesting to examine the interaction between hY5 RNA and human Ro60 under stress conditions, as this RNA may have evolved to aid Ro60 in its nuclear function.

It has been demonstrated that the internal loop region of each Y RNA interacts with a different set of proteins, forming an RNP with a unique function (35, 40, 41). For example, the mouse Y3-Ro60 complex associates with zipcode-binding protein 1 (ZBP1), while the mY1-Ro60 complex does not (42). ZBP1 contains a chromosome region maintenance 1 (CRM1)-dependent signal for nuclear export (43, 44), which could explain why mY3-Ro60 is exported from the nucleus by a CRM1-dependent exporter, while mY1-Ro60 export is not CRM1-dependent (42, 45, 46). As well as alternative export pathways leading to possible differences in location, Y RNAs also have an overlapping but distinct distribution at euchromatic sites in the nucleus (30). These results further indicate that each Y RNA has a localisation-dependent function, as, although there is redundancy in the function of all vertebrate Y RNAs in chromosomal replication, each Y RNA may be involved in the initiation of replication at a particular subset of sites (30). Hogg and Collins (35) proposed that each of the Y RNAs in humans was capable of recruiting a different group of defective RNAs indirectly through interactions with bridging proteins, highlighting potentially specialised functions in RNA quality control as well as chromosomal replication.

A large number of proteins have recently been found to interact with Y RNAs, including many that are involved in chromosomal replication (30, 47, 48). Examining these RNA-protein interactions more closely should lead to further characterisation of their function in chromosomal replication and in other cellular processes. This in turn would enable a better understanding of the extent of Ro60- and La-free Y RNA function.

Y RNA-derived small RNAs

A fascinating feature of the Ro-RNPs is that their RNA component is altered during apoptosis. Shorter RNA fragments are rapidly generated during apoptosis from all human Y RNAs in a caspase-dependent manner (49). There are two kinds of shorter fragments: the smaller

fragment is around 22–25 nucleotides and the longer fragment is around 31 nucleotides (49). Interestingly, these Y RNA-derived small RNAs (YsRNAs) are still bound to proteins: the longer fragment was immunoprecipitated with both Ro60 and La, while the shorter fragment was bound only to Ro60 but not La (49). Although these YsRNAs were first reported in 1999, it took more than 10 years to follow up on these curious molecules. The most likely reasons for this long wait are the uniqueness of these short fragments and the technical difficulty to work with such short RNA molecules.

Coincidentally, the last decade saw a remarkable growth of interest in small non-coding RNAs, mainly because of the discovery of microRNAs (miRNAs) (50–52). miRNAs in animals are mainly 22 nucleotides and are generated from a longer precursor RNA (pre-miRNA) with a characteristic stem-loop structure. The conserved RNaseIII family member Dicer recognises the stem as a partially double-stranded RNA and cleaves it, generating a 20mer duplex RNA with two-nucleotide overhangs at each 3′ end (53). One of the strands is incorporated into an Ago2 complex and is called the mature miRNA, and the other strand, often called the miRNA*, is degraded. The mature miRNA can recognise specific mRNAs on the basis of sequence complementarity and therefore guides the Ago2 complex to target mRNAs. This results in downregulation of the target mRNAs' expression, and it is now generally accepted that miRNAs play an important role in development and many diseases (53).

In addition to the discovery of miRNAs, the arrival of next-generation sequencing (NGS) played an important role in the characterisation of YsRNAs. NGS allowed the identification and profiling of small RNAs on a previously unprecedented scale and as its cost was falling, it was applied to sequence small RNAs by more and more groups. One such study sequenced small RNAs in precursor B cells of acute lymphoblastic leukaemia patients and in normal $CD34^+$ cells and found 24-nucleotide fragments from the 3′ end of Y RNAs (54). However, this study did not realise that these sequences were Y RNA fragments, as there were many thousands of other sequences. Because these sequences formed a stem-loop structure with the flanking genomic sequences, they were predicted to be miRNAs and were named miR-1975 (generated from Y5) and miR-1979 (generated from Y3). In the following year, Meiri et al. (55) also found miR-1975 and miR-1979 in several solid tumours; however, they noticed that these sequences were derived from Y RNAs. At that time, it was already reported that other non-coding RNAs can be processed by Dicer, producing functional miRNAs including tRNAs and snoRNAs (56, 57); therefore, they tested the activity of miR-1975 and miR-1979 using the standard luciferase assay (58). The test proved to be negative; however, a single negative result did not rule out the possibility that Y RNAs were processed by Dicer, leading to functional miRNAs. Nevertheless, miR-1975 and miR-1979 were removed from miRBase, which is the primary database for miRNAs (59), as there was not sufficient evidence that they were miRNAs. However, the question still remained whether Y RNAs can serve as pre-miRNAs and produce functional miRNAs (17).

A recent study focused on this question and first it addressed whether the amount of YsRNAs was in the range of known miRNAs (60). Although, the level of YsRNAs is dramatically increased during apoptosis, they can be detected in non-apoptotic cells where their level is similar to the level of known miRNAs. Next, it was tested whether the generation of YsRNAs is dependent on Dicer activity. A cell line was used where one of the exons of Dicer is deleted, and apoptosis was induced by polyI:C in both wild-type and Dicer knockout cells. The level of Y3 and Y5 sRNAs increased significantly by polyI:C in both wild-type and Dicer knockout cells, indicating that Dicer is not required for the production of YsRNAs (60). Next, Ago2 complexes were immunoprecipitated and the presence of known miRNAs and YsRNAs was investigated by Northern blot analysis. miR-21 was readily detected in the precipitated complex; however, Y3 and Y5 sRNAs were not detectable in the pull-down, demonstrating that YsRNAs are not in complex with Ago2. Finally, lysates of polyI:C-treated cells were separated by anion exchange chromatography and known miRNAs and YsRNAs were detected in the fractions by Northern blot analysis. This experiment revealed that Y3 and Y5 sRNAs were in different fractions to known miRNAs, which supported the previous result that YsRNAs were not in complex with Ago2. On the basis of these results (60), it can now be concluded that Y RNAs do not enter the miRNA pathway and the YsRNA fragments do not act as miRNAs.

The future of Y RNA research

The RNA 'Zoo' within the cell, and the interactions these RNAs have with other cellular components, appears to be more complex than first thought. Indeed, it is sometimes misleading to think of each RNA family as a discrete group of molecules, as there can be significant interchangeability between groups. For instance, some transfer RNAs (tRNAs) and small nucleolar RNAs (snoRNAs) can enter small interfering RNA (siRNA)-like pathways (61, 62).

Further to this, the number of small RNAs derived from longer RNA molecules also appears to be a significant and emerging field in its own right, with several papers and reviews already appearing in this area (60, 63–67).

Although Y RNAs were first identified >30 years ago (6), only a relatively small number of papers have been published about their actual biological function, with most of the literature documenting their structure, RNA-protein interactions, and general biochemistry. A major biological function of a fraction of Y RNAs appears to be in chromosomal DNA replication. As the role of Y RNAs in DNA replication does not require Ro60, it is likely that Ro60 regulates the availability of Y RNAs to execute this function by regulating their stability. Future work should focus on the exact molecular mechanism by which Y RNAs initiate DNA replication in post-MBT cells.

Furthermore, transfection of siRNAs against hY1 (25) and hY3 (68) into a range of different cells, including carcinoma cells (including bladder, prostate, cervical, and lung) results in a reduction in cell proliferation. This makes Y RNAs promising targets for future pharmacological treatments in cancer patients.

The appearance of Y RNA-derived sRNAs, and indeed similar-sized sRNAs derived from other non-coding RNAs in a number of studies (26, 49, 55, 60), implies that these molecules may support a biological pathway that has yet to be uncovered. Y RNA-derived sRNAs may modulate gene expression at the transcriptional level in a similar fashion to how sRNAs derived from tRNAs modulate translation (65), and future investigations should try and address this hypothesis. Another exciting question is how these Y RNA-derived sRNAs are produced. One possibility is that a specific enzyme cleaves the Y RNAs, producing the smaller fragments. However, it is also possible that the Y RNAs are degraded by non-specific RNases and the proteins bound to them protect the regions that have been identified as Y RNA-derived sRNAs. In the first scenario, it is essential to identify the enzyme that cleaves Y RNAs and if the second possibility is true it will be important to clarify which proteins protect those specific regions.

Finally, a variety of sRNAs have been sequenced in a population of 'shuttle RNAs' present in the vesicles released by immune cells (64). RNA cleavage products derived from vault RNAs, tRNAs and Y RNAs were detected, whereas miRNAs were significantly underrepresented in the shuttle RNA population. Y RNAs were some of the most abundant RNAs present in the immune vesicles, with both 28-nucleotide fragments and full-length Y RNAs being detected. The similarity by which Y RNAs were assimilated into immune cell-derived vesicles (64), and the nature by which they are selectively packaged into viruses, which was reported previously (69), is worth noting. Future studies will clarify whether the Y RNAs could play a role in sorting of regulatory RNAs into these vesicles, in stabilising other RNAs during export, or in guiding shuttle RNAs to specific locations in vesicle-targeted cells (64).

Received November 14, 2012; accepted December 5, 2012

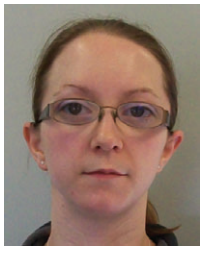
References

1. Sharp PA. The centrality of RNA. Cell 2009; 136: 577–80.
2. Gilbert W. Origin of life – the RNA world. Nature 1986; 319: 618.
3. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet 2006; 15: R17–29.
4. Birney E. ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447: 799–816.
5. Lerner MR, Steitz JA. Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. Proc Natl Acad Sci USA 1979; 76: 5495–9.
6. Lerner MR, Boyle JA, Hardin JA, Steitz JA. Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. Science 1981; 211: 400–2.
7. Mosig A, Guofeng M, Stadler BM, Stadler PF. Evolution of the vertebrate Y RNA cluster. Theory Biosci 2007; 126: 9–14.
8. Perreault J, Perreault JP, Boire G. Ro-associated Y RNAs in metazoans: evolution and diversification. Mol Biol Evol 2007; 24: 1678–89.
9. Wolin SL, Steitz JA. The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. Proc Natl Acad Sci USA 1984; 81: 1996–2000.
10. O'Brien CA, Wolin SL. A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5S rRNA precursors. Genes Dev 1994; 8: 2891–903.
11. O'Brien CA, Harley JB. A subset of hY RNAs is associated with erythrocyte Ro ribonucleoproteins. EMBO J 1990; 9: 3683–9.
12. Hendrick JP, Wolin SL, Rinke J, Lerner MR, Steitz JA. Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. Mol Cell Biol 1981; 1: 1138–49.
13. Pruijn GJ, Slobbe RL, van Venrooij WJ. Analysis of protein-RNA interactions within Ro ribonucleoprotein complexes. Nucleic Acids Res 1991; 19: 5173–80.

14. Fouraux MA, Bouvet P, Verkaart S, van Venrooij WJ, Pruijn GJ. Nucleolin associates with a subset of the human Ro ribonucleoprotein complexes. J Mol Biol 2002; 320: 475–88.
15. Peek R, Pruijn GJ, van der Kemp AJ, van Venrooij WJ. Subcellular distribution of Ro ribonucleoprotein complexes and their constituents. J Cell Sci 1993; 106: 929–35.
16. Gendron M, Roberge D, Boire G. Heterogeneity of human Ro ribonucleoproteins (RNPS): nuclear retention of Ro RNPS containing the human hY5 RNA in human and mouse cells. Clin Exp Immunol 2001; 125: 162–8.
17. Verhagen APM, Pruijn GJM. Are the Ro RNP-associated Y RNAs concealing microRNAs? Y RNA-derived miRNAs may be involved in autoimmunity. Bioessays 2011; 33: 674–82.
18. Chen X, Wolin SL. The Ro 60 kDa autoantigen: insights into cellular function and role in autoimmunity. J Mol Med 2004; 82: 232–9.
19. Wolin SL, Cedervall T. The La protein. Annu Rev Biochem 2002; 71: 375–403.
20. Takeda D, Dutta A. DNA replication and progression through S phase. Oncogene 2005; 24: 2827–43.
21. Pospiech H, Grosse F, Pisani FM. The initiation step of eukaryotic DNA replication. Subcell Biochem 2010; 50: 79–104.
22. Rao P, Johnson R. Mammalian cell fusion. Studies on regulation of DNA synthesis and mitosis. Nature 1970; 225: 159–64.
23. Krude T, Jackman M, Pines J, Laskey RA. Cyclin/Cdk-dependent initiation of DNA replication in a human cell-free system. Cell 1997; 88: 109–19.
24. Krude T. Initiation of chromosomal DNA replication in mammalian cell-free systems. Cell Cycle 2006; 5: 2115–22.
25. Christov CP, Gardiner TJ, Szuts D, Krude T. Functional requirement of noncoding Y RNAs for human chromosomal DNA replication. Mol Cell Biol 2006; 26: 6993–7004.
26. Gardiner TJ, Christov CP, Langley AR, Krude T. A conserved motif of vertebrate Y RNAs essential for chromosomal DNA replication. RNA 2009; 15: 1375–85.
27. Langley AR, Chambers H, Christov CP, Krude T. Ribonucleoprotein particles containing non-coding Y RNAs, Ro60, La and nucleolin are not required for Y RNA function in DNA replication. PLoS One 2010; 5: e13673.
28. Wolin SL, Steitz JA. Genes for two small cytoplasmic Ro RNAs are adjacent and appear to be single-copy in the human genome. Cell 1983; 32: 735–44.
29. Krude T, Christov CP, Hyrien O, Marheineke K. Y RNA functions at the initiation step of mammalian chromosomal DNA replication. J Cell Sci 2009; 122: 2836–45.
30. Zhang AT, Langley AR, Christov CP, Kheir E, Shafee T, Gardiner TJ, Krude T. Dynamic interaction of Y RNAs with chromatin and initiation proteins during human DNA replication. J Cell Sci 2011; 124: 2058–69.
31. Collart C, Christov CP, Smith JC, Krude T. The midblastula transition defines the onset of Y RNA-dependent DNA replication in Xenopus laevis. Mol Cell Biol 2011; 31: 3857–70.
32. Shi H, OBrien CA, VanHorn DJ, Wolin SL. A misfolded form of 5S rRNA is complexed with the Ro and La autoantigens. RNA 1996; 2: 769–84.
33. Labbe JC, Hekimi S, Rokeach LA. The levels of the RoRNP-associated Y RNA are dependent upon the presence of ROP-1, the Caenorhabditis elegans Ro60 protein. Genetics 1999; 151: 143–50.
34. Chen X, Smith JD, Shi H, Yang DD, Flavell RA, Wolin SL. The Ro autoantigen binds misfolded U2 small nuclear RNAs and assists mammalian cell survival after UV irradiation. Curr Biol 2003; 13: 2206–11.
35. Hogg JR, Collins K. Human Y5 RNA specializes a Ro ribonucleoprotein for 5S ribosomal RNA quality control. Genes Dev 2007; 21: 3067–72.
36. Fuchs G, Stein AJ, Fu C, Reinisch KM, Wolin SL. Structural and biochemical basis for misfolded RNA recognition by the Ro autoantigen. Nat Struct Mol Biol 2006; 13: 1002–9.
37. Stein AJ, Fuchs G, Fu C, Wolin SL, Reinisch KM. Structural insights into RNA quality control: the Ro autoantigen binds misfolded RNAs via its central cavity. Cell 2005; 121: 529–39.
38. Green CD, Long KS, Shi H, Wolin SL. Binding of the 60-kDa Ro autoantigen to Y RNAs: evidence for recognition in the major groove of a conserved helix. RNA 1998; 4: 750–65.
39. Sim S, Weinberg DE, Fuchs G, Choi K, Chung J, Wolin SL. The subcellular distribution of an RNA quality control protein, the Ro autoantigen, is regulated by noncoding Y RNA binding. Mol Biol Cell 2009; 20: 1555–64.
40. Fabini G, Raijmakers R, Hayer S, Fouraux MA, Pruijn GJ, Steiner G. The heterogeneous nuclear ribonucleoproteins I and K interact with a subset of the ro ribonucleoprotein-associated Y RNAs in vitro and in vivo. J Biol Chem 2001; 276: 20711–8.
41. Bouffard P, Barbar E, Briere F, Boire G. Interaction cloning and characterization of RoBPI, a novel protein binding to human Ro ribonucleoproteins. RNA 2000; 6: 66–78.
42. Sim S, Yao J, Weinberg DE, Niessen S, Yates JR, III, Wolin SL. The zipcode-binding protein ZBP1 influences the subcellular location of the Ro 60-kDa autoantigen and the noncoding Y3 RNA. RNA 2012; 18: 100–10.
43. Nielsen J, Adolph S, Rajpert-De Meyts E, Koch G, Christiansen J, Nielsen F. Nuclear transit of human zipcode-binding protein IMP1. Biochem J 2003; 376: 383–91.
44. Oleynikov Y, Singer R. Real-time visualization of ZBP1 association with β-actin mRNA during transcription and localization. Curr Biol 2003; 13: 199–207.
45. Gwizdek C, Ossareh-Nazari B, Brownawell A, Doglio A, Bertrand E, Macara I, Dargemont C. Exportin-5 mediates nuclear export of minihelix-containing RNAs. J Biol Chem 2003; 278: 5505–8.
46. Rutjes SA, Lund E, van der Heijden A, Grimm C, van Venrooij WJ, Pruijn GJ. Identification of a novel cis-acting RNA element involved in nuclear export of hY RNAs. RNA 2001; 7: 741–52.
47. Sim S, Wolin SL. Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. Wiley Interdiscip Rev RNA 2011; 2: 686–99.
48. Koehn M, Lederer M, Waechter K, Huettelmaier S. Near-infrared (NIR) dye-labeled RNAs identify binding of ZBP1 to the noncoding Y3-RNA. RNA 2010; 16: 1420–8.
49. Rutjes SA, van der Heijden A, Utz PJ, van Venrooij WJ, Pruijn GJ. Rapid nucleolytic degradation of the small cytoplasmic Y RNAs during apoptosis. J Biol Chem 1999; 274: 24799–807.
50. Lee R, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001; 294: 862–4.
51. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001; 294: 858–62.
52. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294: 853–8.

53. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–97.
54. Schotte D, Chau JCK, Sylvester G, Liu G, Chen C, van der Velden VHJ, Broekhuis MJC, Peters TCJM, Pieters R, den Boer ML. Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia 2009; 23: 313–22.
55. Meiri E, Levy A, Benjamin H, Ben-David M, Cohen L, Dov A, Dromi N, Elyakim E, Yerushalmi N, Zion O, Lithwick-Yanai G, Sitbon E. Discovery of microRNAs and other small RNAs in solid tumors. Nucleic Acids Res 2010; 38: 6234–46.
56. Ender C, Krek A, Friedlaender MR, Beitzinger M, Weinmann L, Chen W, Pfeffer S, Rajewsky N, Meister G. A human snoRNA with microRNA-like functions. Mol Cell 2008; 32: 519–28.
57. Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JWS, Green PJ, Barton GJ, Hutvagner G. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. RNA 2009; 15: 2147–60.
58. Lewis B, Shih I, Jones-Rhoades M, Bartel D, Burge C. Prediction of mammalian microRNA targets. Cell 2003; 115: 787–98.
59. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39: D152–7.
60. Nicolas FE, Hall AE, Csorba T, Turnbull C, Dalmay T. Biogenesis of Y RNA-derived small RNAs is independent of the microRNA pathway. FEBS Lett 2012; 586: 1226–30.
61. Pederson T. Regulatory RNAs derived from transfer RNA? RNA 2010; 16: 1865–9.
62. Brameier M, Herwig A, Reinhardt R, Walter L, Gruber J. Human box C/D snoRNAs with miRNA like functions: expanding the range of regulatory RNAs. Nucleic Acids Res 2011; 39: 675–86.
63. Li Z, Ender C, Meister G, Moore PS, Chang Y, John B. Extensive terminal and asymmetric processing of small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. Nucleic Acids Res 2012; 40: 6787–99.
64. Nolte-'t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, 't Hoen PA. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. Nucleic Acids Res 2012; 40: 9272–85.
65. Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P. Angiogenin-induced tRNA fragments inhibit translation initiation. Mol Cell 2011; 43: 613–23.
66. Rother S, Meister G. Small RNAs derived from longer non-coding RNAs. Biochimie 2011; 93: 1905–15.
67. Tuck AC, Tollervey D. RNA in pieces. Trends Genet 2011; 27: 422–32.
68. Christov CP, Trivier E, Krude T. Noncoding human Y RNAs are overexpressed in tumours and required for cell proliferation. Br J Cancer 2008; 98: 981–8.
69. Garcia EL, Onafuwa-Nuga A, Sim S, King SR, Wolin SL, Telesnitsky A. Packaging of host mY RNAs by murine leukemia virus may occur early in Y RNA biogenesis. J Virol 2009; 83: 12526–34.
70. van Gelder CW, Thijssen JP, Klaassen EC, Sturchler C, Krol A, van Venrooij WJ, Pruijn GJ. Common structural features of the Ro RNP associated hY1 and hY5 RNAs. Nucleic Acids Res 1994; 22: 2498–506.

Adam E. Hall studied Biological Sciences at the University of Birmingham and later went on to specialise in Molecular Genetics at the University of Leicester for his master's degree. He then moved to Norwich for his PhD to work on small non-coding RNAs. Specifically, he has been investigating microRNAs and Y RNAs in animal cells.



Carly Turnbull studied for a BSc in Genetics at the University of Leicester before working for the NHS West Midlands Regional Genetics Service. Prior to her PhD, she undertook research assistant positions at the MRC/CSC Genomics Laboratory in London and at the Weatherall Institute of Molecular Medicine in Oxford. She is now in her fourth year of a PhD focusing on the cleavage of human Y RNAs.

Tamas Dalmay graduated in Budapest and obtained his PhD from the Hungarian Academy of Science. He moved to The Sainsbury Laboratory in 1995 where he worked on the genetics of gene silencing in plants with Prof. David Baulcombe. He established his group in 2002 at the University of East Anglia, Norwich, UK. His group focuses on the biology of small regulatory RNAs in plants and animals.