Editorial

Nuclear export mechanisms of circular RNAs: size does matter

Asfar S. Azmi

Department of Oncology, Wayne State University School of Medicine, Detroit, MI 48201, USA

Correspondence to: Asfar S. Azmi, PhD. Department of Oncology, Wayne State University School of Medicine, 4100 John R, HWCRC 732, Detroit, MI 48201, USA. Email: azmia@karmanos.org.

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Non-coding RNAs (ncRNAs) are broadly classified as house-keeping and regulatory ncRNAs. The house keeping ncRNAs include the ribosomal RNA, transfer RNA, small nuclear RNA and snoRNA. While the regulatory ncRNA classification is based on length and include shorter microRNAs, snRNAs, piRNAs or the long ncRNAs (lncRNAs) that are greater than 200 nucleotides (nt) (1). Circular RNAs (circRNAs) are a unique class of ncRNAs that have covalently linked ends and fall under the lncRNA group. Their hidden 3' ends make them resistant to endonuclease attack and this in turn allows for their accumulation in cells (2). Studies indicate that circRNAs arise when the pre-mRNA splicing apparatus undergoes a phenomenon of back splicing. This results in joining of the 2' exon of a splice donor to the 2' start of the splice acceptor (3). The field of circRNAs is understudied and relatively new. There is sporadic information emerging on their implications in diseases such as cancer. Their role in cell physiology is not clearly known, although studies show that circRNAs can modulate the function of several microRNAs by acting as sponges (4). There are some insights on the biogenesis of circRNAs (5), however, how these are spatially distributed and what is the mechanism of their transport within the cell was not known until very recently. Investigations in this area are critical since circRNAs are synthesized in the nucleus but are found to be mostly localized in the cytoplasm. Their preferential cytoplasmic localization indicates that these ncRNAs are somehow transported across the nuclear membrane. A recent Genes and Development article by Huang *et al.* (6), sheds light into the nuclear export mechanisms regulating the distributions of these unique RNAs.

With the emergence of nucleus in eukaryotic cells came the need for effective mechanism to transport biomolecules to and from the cytosol. Cells devised specialized transporters to conduct such fluid exchange of biomolecules through the nuclear pore complex to maintain the proper sub-cellular compartmentalization of proteins and RNA (7). The Karyopherin family proteins (importins and exportin/XPO1) are the main nuclear importer and exporter of proteins. The transport of nuclear export signal (NES) and nuclear localization signal (NLS) carrying proteins is quite well studied and is facilitated by RAN-GAP (in the nucleus) and RAN-GEF (in the cytosol) (8). Small non-coding RNA (microRNA) maturation occurs in the cytosol and requires efficient nuclear export through the karyopherin family member exportin 5 (XPO5) (9). A limited set of studies have also shown that aside from XPO5, the export of few precursor miRNAs occurs through XPO1 as well (10). The mRNAs have a distinct export mechanism that occurs with the help of adaptor serine/arginine rich proteins that promote the recruitment of heterodimeric mRNA export receptor NXF1-NXT1 which facilitates exit through the nuclear pore (11). Despite some available knowledge on microRNA nuclear transport, the non-coding RNA nuclear transport in general remains an under-studied field. More specifically, there is little information available on the nuclear import and export mechanism of ncRNAs including piRNA, lncRNA and circRNAs. Key questions remain as to what is the selection process of nuclear import or export of non-coding RNAs.

Interestingly, the majority of circRNAs are found to be accumulated in the cytosol. What causes their spatial distribution in cells and whether their nuclear export is

through an active transport process was studied by Huang and colleagues in their recent Genes and Development article. Their report sought to decipher whether the nuclear export machinery sorts circRNAs based on the length. Using RNA interference (RNAi for dsRNA) on 26 known nuclear exporters the impact on circRNA localization in cellular nuclear fractions in Drosophila DL1 cells was evaluated. Their initial screen revealed that RNAi against DExH/D-Box helicase Hel25E resulted in nuclear accumulation of circRNAs. More interesting was the observation that Hel25E knockdown mediated nuclear accumulation was restricted to circRNAs with lengths >800 nt. This came to light when the authors showed nuclear accumulation of circRNAs that were >811 nt and not of those <702 nt in the Hel25E RNAi screen. In further set of experiments, the authors generated a series of expression plasmids that could produce circRNAs of different lengths followed by Hel25E RNAi. The results further corroborated their hypothesis as they observed increased reliance on Hel25E mediated export with the enhancement of the circRNA length.

After establishing the role of Hel25E in DL1 models, the authors investigated whether human circRNA nuclear export is also length-dependent. For such studies, human homologs of Hel25E the UAP56/DDX39B (a RNA helicase) and URH49/DDX39A were evaluated. UAP56 and URH49 have been shown to play a significant role in mRNA export and have ~90% similarities to Hel25E with majority of the differences observed in the first 30 amino acids (12). Interestingly, in these experiments, shorter circRNAs were found to be specifically exported by URH49. On the other hand circRNAs >1,200 nt were preferentially exported by UAP56. Further experiments were performed to pinpoint the region within these exporter proteins responsible for regulating the export of circRNAs. A rescue experiment using mutant constructs for different motifs within these two homologs were introduced in cells knockdown with Hel25E (for DL1) or UAP56 (for human cells). These results showed that construct that lacks ATP binding affinity or the helicase activity in Hel25E also lack long circRNA nuclear export ability. Similarly, in human homolog UAP56, a set of 4 conserved amino acids in the ATP binding domain and helicase domain (AK-K/S-L-N present in both Hel25E and UAP56) were identified to be regulating circRNA nuclear export. Most interestingly, inserting construct with these specific amino acids was sufficient for URH49 to export >1,200 nt circRNAs out of the nucleus.

It would have been interesting if the authors went on to further study the domains responsible for selection of shorter circRNAs. Aside from nuclear and cytosolic location, circRNAs have also been observed to be present in extracellular vesicles or exosomes (13). The mechanism guiding circRNA vesicular uptake/packaging have not been studied and is certainly worth pursuing. Recent publications have shown that specific inhibitors of nuclear export (SINE) compounds (selinexor a phase III drug and eltanexor a phase II drug) can promote protein nuclear retention and consequent sensitization of cancer cells by activation of cell death signaling cascade (14). Similarly, SINE compounds can also alter microRNA expression leading to cancer cell growth arrest (15). These observations implicate the role of XPO1 in non-coding RNA biology. Currently our laboratory is studying the impact of XPO1 inhibition on circRNA localization. It would also be worthwhile to investigate the impact of inhibition of other karyopherin family members on circRNA export machinery and its consequence on compartmentalization.

UAP56 has been shown to be constitutively expressed during cell cycle while URH49 is found to be expressed in quiescent cells (16). Additionally, studies have shown that URH49 is over-expressed in rapidly proliferating cells and also prominently observed in several cancers (17). These observations have led to the assumption that URH49 could become a possible therapeutic target. On a related topic, the UAP56 targeted small molecule inhibitors are currently being investigated against herpes virus (18). These studies have shown that such targeted drugs can interfere with the viral stabilization that requires hTRAX re-modeling through UAP56 helicase activity. Whether, such small molecule drugs can be used to targeting circRNA over-expressing cancers remains to be seen.

In summary, studies conducted by Huang and colleagues shed light not only on the proteins regulating circRNA export, they also provide deep insight into the evolutionarily conserved and precise mechanism on the size selection undertaken during such process.

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Footnote

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