Twins take the job

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The exosome, one of the main cellular ribonucleases in eukaryotes, is a multi-subunit complex of deep evolutionary origin. This complex has been extensively characterized in *Saccharomyces cerevisiae*. Given the good conservation of exosome subunit sequences, it was widely accepted that the yeast exosome provided a good model for other eukaryotic species. Recent analysis of higher eukaryotic exosomes reveals some differences challenging this idea. In this issue of *The EMBO Journal*, Tomecki *et al* (2010) and Staals *et al* (2010) provide new insights into the composition and function of the human exosome,

with implications for our understanding on the organization, function and localization of this complex in these cells.

The exosome is responsible for many RNA processing and degradation reactions in the nucleus and cytoplasm (Lykke-Andersen *et al*, 2009). Yeast exosome is composed of a nine subunit ring structure to which Dis3 binds to form the exosome core. Additional co-factors associate to the exosome core in the nucleus and the cytoplasm. Of the 10 core subunits, only Dis3 possesses enzymatic activity with an RNB domain acting as exoribonuclease and a PIN domain

Figure 1 Schematic view of the composition and localization of human versus yeast exosomes. The nine subunit ring structure (exosome ring) is localized in the cytoplasm and nucleus in all eukaryotes. In human cells (left) the exosome ring interacts with the exoribonuclease RRP6 (hRRP6) and two different DIS3 paralogs: hDIS3 or hDIS3L1 (in an exclusive manner). Although hRRP6 is localized in the cytoplasm and nucleus, hDIS3 is mainly found in the nucleus (but excluded from nucleoli), and hDIS3L1 is strictly cytoplasmic. hDIS3 and hDIS3L1 are active exoribonucleases, but only the N-terminal PIN domain of hDIS3 is active (smile). In *S. cerevisiae* (yeast) cells (right), Dis3 protein (yDis3), which possesses exoribonucleolytic and endonucleolytic activity, is present in the nucleus and cytoplasm and Rrp6 (yRrp6) is confined to the nucleus.

endowed with endoribonucleolytic activity (Dziembowski *et al.*, 2007; Lebreton *et al.*, 2008; Schaeffer *et al.*, 2009; Schneider *et al.*, 2009). The PIN domain also mediates the association of Dis3 with the remainder ring (Bonneau *et al.*, 2009). In the nucleus, the yeast exosome core associates with the 3'-5' exoribonuclease Rrp6p (Allmang *et al.*, 1999). A homologous ring structure forms the basis of the human exosome (Liu *et al.*, 2006). Surprisingly, however, the human RRP6 factor (hRRP6, also designated PM/ScI-100) was reported to be present in both the nucleus and cytoplasm (Brouwer *et al.*, 2001; van Dijk *et al.*, 2007), whereas DIS3 was not found in exosome preparations (Chen *et al.*, 2001).

Tomecki *et al.* (2010) and Staals *et al.* (2010) now report that the genome from human beings and some other higher eukaryotes encodes three DIS3 paralogs: hDIS3, DIS3-like 1 (hDIS3L1) and DIS3-like 2 (hDIS3L2). Functional analysis indicates that these proteins have different functions as only hDIS3 complements a yeast *DIS3* deletion. By using two biochemical approaches, the authors show an interaction between the human exosome ring and the co-factors hRRP6, hDIS3 and hDIS3L1. Although interaction of the ring with hDIS3 and hDIS3L1 seems to be mutually exclusive, hRRP6 is present in all the complexes. Interestingly, analysis of the subcellular localizations shows that hDIS3 and hDIS3L1 are distributed differently in cells. hDIS3 is mainly a nuclear protein excluded from nucleoli, with a possible minor cytoplasmic fraction. In contrast, hDIS3L1 is cytoplasmic. hRRP6 localizes primarily in the nucleus, being specifically concentrated in nucleoli, although a weak cytoplasmic signal is also detected (Figure 1). These different subcellular distributions could be indicative of different functions. However, knocking down of hRRP6 leads to overexpression of hDIS3L1, and, likewise, depletion of hDIS3 or hDIS3L1 results in slightly elevated levels of hRRP6, arguing that these enzymes have partially redundant functions.

Analysis of hDIS3L1 and hDIS3 protein sequences indicates that some conserved amino acids essential for the endonucleolytic activity have been substituted in the PIN domain of hDIS3L1. Biochemical studies with hDIS3 and hDIS3L1, purified from transiently transfected or stable cell lines, confirm that both proteins harbour exoribonucleolytic activity but that only the PIN domain of hDIS3 is active as an endonuclease. These results suggest that some RNA metabolic processes may require both exo- and endoribonuclease activities, whereas others would rely only on exonucleolytic degradation. The function of these proteins *in vivo* is addressed by testing the consequence of depleting each factor on the accumulation of several exosomes substrates. This showed that nuclear factors hRRP6 and hDIS3 are involved in the degradation of the nuclear promoter upstream transcripts (PROMPTs) and in the processing of the 5.8S rRNA. In contrast, the cytoplasmic hDIS3L1 degrades cytoplasmic substrates such as the *c-MYC* and *c-FOS* mRNAs and participates in cytoplasmic rRNA decay.

By taking together the data reported in these two works manuscripts, a new picture emerges in which the human exosome appears more complicated than its yeast cousin. Indeed, the division of the nuclear and cytoplasmic functions of Dis3 to two highly related, yet slightly different, protein 'twins' is likely to confer to the human exosome both plasticity and robustness. Rather than settling this issue, the increased exosome complexity observed in human raises new questions: How variable is the exosome organization in distant eukaryotes? What is the function of hDIS3L2? Do the various exosome complexes interact with the same co-factors? Future experiments should provide answers.

Conflict of interest

The authors declare that they have no conflict of interest.

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