mRNA stability and control of cell proliferation

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Most of studies on cell proliferation examine the control of gene expression by specific transcription factors that act on transcriptional initiation. In the last years, it became evident that mRNA stability/turnover, which can be considered the other side of the moon, provides an important mechanism for post-transcriptional control of gene expression.

In eukaryotes, mRNAs are degraded by different pathways such as deadenylation-dependent decapping pathway and the deadenylation-independent decapping pathway or nonsense-mediated decay (NMD), the latter involved in the elimination of aberrant polyadenylated mRNAs.

Many proteins involved in these processes are conserved from bacteria to yeast and humans.

Recent papers showed the involvement of proteins deputed to decapping in controlling cell proliferation, virus replication and cell death. In this paper I will reviewed the newest findings in this field.

Keywords mRNA degradation; apoptosis; yeast

1. mRNA degradation

In eukariotes, cytoplasmic mRNA decay is initiated by removal of poly(A) tail. This first step in the turnover pathway is unique in that it is reversible and some transcripts can be readenylated and return to polysomes. CCR4–NOT is the main deadenylase in *S. cerevisiae* [1] and is a large complex of nine proteins, two of which, Ccr4 and Caf1 (also known as Pop2), have exonuclease domains.

Once the cell determines that an mRNA must be destroyed, it can undertake one of two irreversible routes. One of these is "decapping", a process by which the 5' cap is removed, followed by a degradation in the 5'-3' direction by the Xrn1 exoribonuclease. Messenger RNAs also can be degraded in the opposite direction by the exosome, a protein complex composed of 3′ to 5′ nucleases. These two pathways are not mutually exclusive and their balance varies among mRNAs and organisms.

In the yeast *Saccharomyces cerevisiae*, after the loss of the poly(A) tail the Lsm1p-7p/Pat1p/ Dhh1p complex assembles at the 3' end of the mRNA, possibly recognizing a short oligo(A) tract. Binding of the Lsm1-7 complex may remodel the mRNA structure to allow the decapping enzyme to access the cap, or Lsm proteins may directly recruit the decapping enzymes.

Dcp1/Dcp2 and Xrn1 occur in a larger complex with the Lsm1–7 proteins, all of which colocalize at cytoplasmic foci known as processing (P)-bodies. P-bodies are believed to be the site of decapping and 5'–3' mRNA decay [2,3].

Lsm complexes seem also to be able to inhibit RNA degadation. In fact, loss of Lsm1-7 function results in 3' end trimming of deadenylated mRNA [4] and Lsm proteins have been implicated in stabilizing the 3' end of intermediates in the rRNA [5] and snoRNA [6] processing pathways. These effect on mRNA decay are probably mediated by the interactions of Lsm proteins with various components of the mRNA-decay machinery, including the decapping enzyme (Dcp1-Dcp2), 5'-3' exonucleases (Xrn1, Rat1) and components of the exosome complex, which performs 3'-5' decay [7].

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Although most transcripts undergo deadenylation-dependent decay as described above, there are specific mRNAs that seem to bypass the standard pathways and employ several unusual routes to decay such as the deadenylation-independent decapping [8].

Furthermore, the cell has evolved means for detecting and degrading aberrant transcripts, thereby protecting it from potentially toxic protein products.

Surveillance for inappropriate mRNA processing occurs in the nucleus, whereas three pathways detect aberrant messenger ribonucleoprotein (mRNP) structures in the cytoplasm and are translation dependent. The nonsense-mediated decay (NMD) detects and degrades transcripts that contain premature termination codons (PTCs) arisen from mutations frame-shifts [9]. The non-stop decay (NSD) targets mRNAs that lack a stop codon generated by breakage, or by the absence of an in-frame stop codon [10]. Finally, the last discovered is the no-go decay (NGD) that prevents the sequestration of translation factors to faulty transcripts by detecting stalled ribosomes on an mRNA and endonucleolytically cleaving the mRNA near the stall site. This releases the stalled ribosome and mRNA fragments, which are decayed by the exosome and Xrn1 [11].

In the recent years, dysfunction of mRNA degradation, in particular in the decapping pathway, have been shown to be crucial in cell proliferation, apoptosis and viral propagation.

2. Role of mRNA stability in cell proliferation and apoptosis

Alterations of gene expression have an important role in tumorigenesis. This can be achieved by specific transcription factors that control of transcriptional initiation; nevertheless, it is evident that mRNA turnover provides a further important mechanism for post-transcriptional control of gene expression.
The CaSm yeast homolog, Lsm1, has a critical role in decapping [12] and, although the function(s) of human hLsm1 has not been determined, there are some evidences that also hLsm1p takes part to mRNA degradation process. In fact, components of LSm1-LSm7 complex colocalize with human Dcp1/2 and Xrn1 in discrete foci [13-15] deputed to mRNA degradation [2]. Moreover, antisense-mediated reduction of CaSm is associated with increased p21/CIP mRNA stability [16].

Human Lsm1, also called CaSm (cancer-associated Sm-like) was originally identified based on elevated expression in pancreatic cancer, in several cancer-derived cell lines [17] as well as in metastatic tumors [18].

CaSm is also required to maintain the transformed phenotype of prostate cancer cells and it can function as a oncogene in that over-expression of this gene in NIH-3T3 and MCF10A cells leads to foci formation in vitro [16,19].

The expression of CaSm antisense, as well as the inhibition of CaSm by siRNA, have a clear effect on blocking cell proliferation of cancer cells prior the completion of mitosis. The reduction of CaSm protein lead to lower levels of cyclin B1 and the cyclin dependent kinase CDK1, that are required for the normal G2-M progression of the cell cycle and this can explain the observed cell cycle block. Conversely, CaSm over-expression could increase CDK1 function in cancer cells, causing the bypass the G2 checkpoint and an increase in proliferation rate.

Although apoptotic cells were not observed during the Ad-anti-CaSm treatment, it could be hypothesized that apoptosis could occur in the absence of hLsm1.

In fact, it has been clearly described that the reduction of cyclin B1 causes inhibition of proliferation by arresting cells in G2 phase and by inducing apoptosis in HeLa cells [20]. It can be that cells infected with anti-CaSm-expressing virus only reduce, but not completely abolish, the hLsm1 function.

The Dhh1 (RCK/p54, Me31B) protein, a DExD/H-box RNA helicase, is an important component of decapping machinery that is associated to the cytoplasmic P-bodies.

Also the inappropriate regulation of RCK/p54 expression has been implicated in many types of tumors and in precancerous conditions, such as hepatitis C infection [21-23].

In colon tumour cells, the overexpression of rck/p54 is frequently accompanied by over-expression of c-Myc, suggesting that rck/p54 contributes to the stabilization and increased translational efficiency of c-myc mRNA.

The complete knock out of a human gene is not so easy, and the use of other simpler systems, like yeast, can be useful to unravel complex networks.

The yeast *Saccharomyces cerevisiae* is a simple eukaryotic organism with just approximately 6000 genes. The genome sequence is known, more than 60% of the genes have an assigned function, while more than 40% share conserved sequences with at least one known or predicted human gene. Moreover, yeast is easy to grow in culture and to manipulate genetically.

Recent studies have established yeasts as models to study mechanisms of apoptotic regulation. The yeast *Saccharomyces cerevisiae* shows cell death with typical markers of apoptosis, such as DNA fragmentation, phosphatidylserine externalization and chromatin condensation in a strain carrying a mutation in the AAA-ATPase gene *CDC48* [24]. VCP, its mammalian orthologue, was subsequently linked to the regulation of apoptosis [25]. Moreover, heterologous expression of the pro-apoptotic protein hBax induces apoptosis in yeast cells and this can be counteracted by the simultaneous expression of the anti-apoptotic protein Bcl-2.

In addition, exposure to low doses of H_2O_2 or acetic acid, that are known to increase ROS production, induces apoptosis in wild type yeast cells, indicating that, like in metazoans, ROS are a key regulator of yeast apoptosis [26,27].

Several homologues of classical apoptotic regulators were identified and characterized in yeast. Advanced pattern based sequence homology search led to the identification of metacaspase *YCA1* in *S. cerevisiae* [28]. Yca1p has a central role in yeast apoptosis, characterizing it as an orthologue of mammalian caspases. In fact, mutants in *YCA1* gene show decrease of cell death under oxygen stress and during aging.

Yeast mutants in genes involved in decapping (*lsm1*, *lsm4*, *lsm6*, *lsm7*, *dcp1*, *dcp2*, *dhh1* and *pat1*) show accumulation of mRNA degradation intermediates and accumulation of intracellular ROS [29]. These mutants undergo cell death prematurely during stationary phase and show the typical markers of apoptosis [29]. Most of the cell death observed in one of these mutants (*lsm4*) was rescued by deletion of the *YCA1* gene, that codes for a meta-caspase in yeast (madeo caspasi), suggesting that caspase activity is required for apoptosis induced by increased mRNA stability [30].

The bacterial Hqf protein, the prokaryotic Lsm counterpart, forms a homoexameric doughnut-complex and, as the eukaryotic Lsm proteins, take part to many aspect of RNA life. As Lsm complex, it can stabilize RNAs or promote their degradation.

In *E. coli*, Hfq inactivation caused pronounced pleiotropic phenotypes, including decreased growth rates and yields, decreased negative supercoiling of plasmids in stationary phase, increased cell size, osmosensitivity, increased oxidation of carbon sources, increased sensitivity to ultraviolet light [31] and loose viability during heat shock [32].

Moreover, Hfq is essential for *Vibrio cholerae* and *P.aeruginosa* virulence [33,34] and also fundamental for oxidative stress resistance and stationary phase in *Salmonella typhimurium* [35].

It is clear that at least some phenotypes, are in common with yeast *lsm* mutants [36,37] suggesting a strong conservation of these proteins through evolution.

3. Role of the Lsm1p-7p/Pat1p/Dhh1p complex in viral replication

Yeast was also used as a model in the study of virus life cycles including virus–host interactions. To this purpose, systems were developed to replicate viruses with RNA and DNA genomes that infect plants, animals and humans, in the yeast *Saccharomyces cerevisiae*.

The first higher eukaryotic virus reported to replicate in yeast was *Brome mosaic virus*, a positive-strand RNA ((+)RNA) virus that infects plants [38].

The genome of BMV consists of three genomic RNAs with 5' caps and tRNA-like 3' ends [39]. RNA1 and RNA2 encode the essential RNA replication factors 1a and 2a that direct BMV RNA replication in *Saccharomyces cerevisiae* [38], reproducing the known features of BMV replication in plants [40].

Among the identified genes that facilitate BMV RNA replication, four of them belong to the complex involved in mRNA decapping (*LSM*1, *LSM*6, *PAT*1 and *DHH*1). These genes play a key role in the

regulated transition of the BMV RNA from the cellular translation machinery to the site of replication [41,42]. As described above, the Lsm1p-7p/Pat1p/Dhh1p complex is involved the movement of mRNAs out of the translating polysome pool, into P bodies, where they will be degraded or stored. This transition from a translation-competent status to a degrading-competent form, requires dramatic rearrangements in the state of the mRNA, including a loss of ribosomes and translation factors and the addition of mRNA decapping factors.

As positive-strand RNA genomes mimic cellular mRNAs, the Lsm1p-7p/Pat1p/Dhh1p complex could mediate rearrangements in the BMV RNA that would facilitate the loss of ribosomes and translation factors and the recognition of the 1a replicase.

Interestingly, Hfq is required for replication of the Q bacteriophage, a (+)RNA virus [43] indicating once more that Lsm1p-7p/Pat1p/Dhh1p has functions that are conserved from prokaryotes to eukaryotes and is utilized by a plant as well as a bacteriophage (+)RNA virus.

4. Conclusions

In conclusions, the perturbations of mRNA degradation can influence cell proliferation/death and viral replication in both eukaryotic and prokaryotic cells. The complex mechanisms that link the amount of mRNAs to cell proliferation are still largely unknown and future works are needed to clarify which are the signals involved in these processes.

The unicellular yeast *Saccharomyces cerevisiae* is a proven model eukaryote for molecular and cellular biology studies and, with the development of genomic technologies, genome-wide approaches could help in the identification of novel molecular mechanisms and their participating proteins.

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