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Review Article

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Special mRNAs exported slowly the reasons yet to uncover in budding yeast.

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Abstract

Special mRNAs specially *SKS1* do not show abnormalities like 5'capping defects and 3'polyadenylation defects. These intronless special mRNAs are used to retain within the nucleus and transported slowly from the nucleus to the cytoplasm. Due to slow movement from the nucleus to the cytoplasm, the special mRNAs do not escape from the nuclear degradation. Within the nucleus, special mRNAs are degraded by the components of nuclear degradation machinery, *CBC1*, and *RRP6*. Significant stability of the special mRNAs have been established in *CBC1* and *RRP6* deleted strain. On the other hand, typical mRNAs like ACT1, CYH2, etc., show normal movement which is faster than the special mRNAs and escape the nuclear degradation machinery. A kinetic competition is also present between the special mRNAs and the typical mRNAs. Molecular dissection by Das et al. revealed a zipcode sequence within the SKS1 transcript body thought to responsible for the slower movement of the special mRNAs. In this context, molecular dissection of the other special mRNAs was needed to corroborate the zipcode sequence concept for the special mRNAs. In this review, a comprehensive visualization has been made regarding the causes of slower export of mRNA, especially special mRNAs.

Keywords: Nuclear degradation machinery (NDM), Zipcode, Special mRNA, Typical mRNA, Quality control.

Introduction

After synthesis, processing, and RNP assembly the mRNAs are exported out of the nucleus from the site of synthesis to the cytoplasm to mostly take part in protein synthesis machinery (4, 14). mRNAs that are unable to export remain inside the nucleus. These export inefficient mRNAs are retarded within the nucleus and destroyed by some nuclear degradation machinery (NDM) (1). Specific defects within the transcript body such as 5'capping defect, splicing defect, 3' cleavage and polyadenylation defects make an mRNA export inefficient and these mRNAs are not recognized by the protein factors thought to involve in mRNA transportation. This has been established with elegant studies of mutants that are defective in pre mRNA maturation. (10,55,12,13,14). These defective mRNAs or export inefficient mRNAs are the burden of the cell as they do not take part in the protein synthesis machinery. In other words, if the aberrant mRNAs take part in the

protein synthesis machinery they will produce some deleterious product which may induce some disease conditions. Many surveillance mechanisms are present within the nucleus to look after the quality of mRNAs. Components of NDM detect the defective mRNAs and destroy them within the nucleus. On the other hand, the normal typical mRNAs do not have such processing defects that will be exported out of the nucleus and save themselves from nuclear degradation. In this regard, a question is emerging whether any normal mRNAs will be retained within the nucleus for the sake of gene expression. A clue to this question came from the studies by Kuai et al. He demonstrated that some normal special kinds of mRNAs are also retarded within the nucleus (1). As there are no so-called mRNAs processing defects that have been identified within these normal special kinds of mRNAs, these are not detected and degraded by the nuclear degradation machinery (1). The next key question is whether cell getting any advantages from retarded normal mRNAs. It has been found that normal mRNAs retarded within the nucleus do not stay for a longer period (1). They are also exported to the cytoplasm. But their export rate is slow. Das et al., have demonstrated that a cis-acting mRNA element within the mRNAs transcript body thought to responsible for slower export of the special mRNAs (2). They also established that elimination of the cis-acting element makes the special mRNA as that of typical mRNA and exported out of the nucleus without being retained within the nucleus (2). The delayed export of the special mRNA arises a couple of questions. Firstly, whether the same set of protein factors are involved in transporting both special kind mRNAs and typical mRNAs. Secondly, the kinds of modifications the export machinery achieve. Thirdly, Is the slow export of an mRNA tightly linked with gene expression? In this review, we try to uncover the possible reasons for the slow export of the normal mRNAs. We also try to investigate whether the special mRNAs are really slow or any other factors masking the real nature of the special mRNAs.

Special mRNA versus typical mRNA: Special mRNAs as named after Kuai et al. show distinct features other than typical mRNAs in respect of mRNA transport. Preferential retention of the special mRNAs within the nucleus gives us the clue for a new avenue of gene expression in budding yeast. Das et al. demonstrated that *CBC1* and *RRP6* gene regulates the abundance of the special mRNAs in the nucleus. They performed a genome-wide screening strategy taking the mRNA transcripts whose steady-state levels and half-lives increased in cbc1-Δ and rrp6-Δ mutant strains to know the susceptible substrates of the *CBC1* and *RRP6* genes (1). They determined the decay rate of individual mRNA in these mutant strains from hybridization intensities at three-time points after the addition of thiolutin considering the mRNA degradation as a single-order reaction (3). Their microarray result initially revealed 500 mRNAs showed increased half-lives in the cbc1-Δ strain. To exclude the unreliable data and possible false-positive they applied a couple of stringent criteria. After the application of the stringent criteria, most of these mRNAs were eliminated and revealed only six normal mRNAs showed increased stability and abundance in the cbc1-Δ and rrp6-Δ mutant strains. Certainly, their stringent criteria eliminated out some mRNAs that were also truly susceptible to nuclear degradation machinery. They further confirmed their microarray result by northern blot analysis. The six mRNAs that came out of their extensive investigation are *SKS1*, *IMP3*, *YLR194C*, *ACT1*, *CYH2*, and *cyc1-512* (1). The half-life of representative special mRNA *SKS1* is 10 min in the normal strain and has been increased to 25 min in cbc1-Δ strain. The other special mRNAs, *IMP3* and *YLR194c* are also similarly stabilized by cbc1-Δ with a 3- to 4-fold elevation in half-life. Interestingly the three typical mRNAs *ACT1*, *CYH2*, and *CYC1* are not stabilized in cbc1-Δ and rrp6-Δ mutant strains (3).

Typical mRNAs, such as *CYC1*, *CYH2*, and *ACT1* mRNAs do not show remarkable differences in stability in export defective strain nup116-Δ as

well as in strain defective both in export and nuclear cap-binding gene, nup116-Δ cbc1-Δ at the permissive temperature, 25^0C. At this permissive temperature, NPC remains opened and mRNAs do not feel any difficulty in moving out of the nucleus and escape from the nuclear degradation machinery pathway. But in the restrictive temperature, at 37^0C the typical mRNAs rapidly degraded in the nup116-Δ strain as the export is blocked. The typical mRNAs also get stabilized in the nup116-Δcbc1-Δ strain as the strain defective both in export and nuclear degradation. Surprisingly the *SKS1*, *IMP3*, and *YLR194C* mRNAs undergo rapid decay both in permissive and restrictive temperature and their rapid degradation is stabilized in a strain defective both in export and nuclear degradation. The data indicate that unlike typical mRNAs the decay rate of special mRNA is independent of export defects at all conditions. Their investigation concluded that special mRNAs such as *SKS1* show preferential nuclear retention which makes them special from typical mRNAs (1,2,3).In this regard, the question arises, how are the special mRNAs getting preference over typical mRNAs in retaining within the nucleus?

Table.1: Details of half life (in min) of mRNAs in different experimental condition.

Representative mRNA	Nature of mRNA	Normal	*CBC1* mutant(inactive NDM)	Fold Change	*Rrp6 mutant(* Inactive NDM*)*	Fold Change
SKS1	Special	10	25	2.5	23	2.3
IMP3	Special	<3	10	>3	14	4.6
YLR194C	Special	5	22	4.4	25	5
ACT1	Typical	34	44	1.3	39	1.15
CYH2	Typical	18	22	1.2	24	1.33

After Kuai et al.,2005

Table 2: Details of half-life(in min) of mRNAs in different experimental condition

mRNAs	Temp (^{o}C)	Normal	*CBC1* del	*RRP6* del	Temp (^{o}C)	Nup 116 del	Nup 116 del cbc1 del	Temp (^{o}C)	Nup116 del	Nup 116 del cbc1 del
SKS1	30	10	25	23	25 Export block is non-functional	20	40	37 Export block is functional	8	20
IMP3		<3	10	14		12	28		<5	12
YLR194C		5	22	25		15	32		9	26
ACT1		34	44	39		42	45		14	52
CYH2		18	22	24		35	38		10	28

After Kuai et al., 2005 (CBC1 & RRP6 deletion mean no decay or degradation of mRNAs.Nup116 deletion means nuclear pore complex is blocked and no mRNAs will be exported out of the nucleus to the cytoplasm)

Overview of the mRNA transport: Unlike small RNAs, the nucleocytoplasmic transportation of mRNA is not dependent on the karyopherin-based mechanism (113). Different upstream and downstream events play a pivotal role in the successful transportation of the mRNAs. Maturation events such as 5'capping, splicing, 3'cleavage, and polyadenylation and recruitment of different export factors make an mRNA export competent. The nuclear export of mRNA transcript can be categorized into distinct stages: first, pre-mRNA is transcribed in the nucleus, where it is matured and packaged into messenger ribonucleoprotein (mRNP) complexes; second, the mRNPs are translocated through nuclear pore complexes (NPC); and third, the mRNPs are directionally released into the cytoplasm (4). So, faithful nuclear processing, recruitment of export factors, and remodeling of the mRNP finally push an mRNA from the nucleus to the cytoplasm through NPC. Any defect in the nuclear processing of an mRNA can be identified by the nuclear surveillance machinery, retained the defective mRNAs in the nucleus and degraded by some nuclear degradation machinery (10,55,12,13,14).

In *Saccharomyces cerevisiae,* a good number of protein factors have already been identified that partaking in coordinated nucleocytoplasmic transportation of mRNAs. It is demonstrated that there are distinct export mechanisms for the different classes of RNAs (102,103). It is reported that though both mRNAs and spliceosomal U snRNAs are produced by RNA polymerase II, they do not follow the same export pathway (102). Little is known about which export pathway governing the slow export of the special mRNAs. It is presumed that a new set of protein factors might be involved in the slower export of the mRNAs. An important question concerns whether these new sets of protein factors will interact with the NPC associated protein in the same manner the other mRNAs do. Proteomic analysis revealed that NPC consist of about 30 distinct protein termed as nucleoporins or Nups of which mostly are stationary in nature and few of them are mobile that facilitates nucleocytoplasmic transport of mRNAs. Nups show octagonal symmetry and varies from 8 to 56 copies per pore (104-106).Furthermore it is found that multiple phenylalanine-glycine(FG) repeat domains situated within NPC conspire for the successful docking of the nucleocytoplasmic transport factor (107). Like protein, t-RNA and micro RNA, the mRNA transport directionality is not determined by a gradient of the GTP bound state of the small GTPase Ran. (4,108). Highly dynamic rearrangement of mRNA-binding and modifying protein factors are needed to eliminate the nuclear retention factor and the addition of signals required for the interaction with the export receptor. (104,109).It is demonstrated that Mex67-Mtr2 heterodimer is recruited initially to the pre-mRNA via the adaptor protein Yra1and sub2 to form the export competent mRNP (110-112). Adapter protein basically establishes a physical bridge between the mRNA transcripts and its export receptor (104,109). It is suggested that Mex67-Mtr heterodimer interacts with FG nucleoporins to equilibrate the nucleocytoplasmic transportation of the mRNAs. At the cytoplasmic face of NPC, the export complex releases Mex67-Mtr heterodimer and get remodeled and thereby prevents mRNAs from returning to the nucleus (113). Another layer of quality control is governed by nuclear NPC associated filamentous protein Mlp1-Mlp2 by retaining intron-containing transcript within the nucleus. (115-117). Several lines of evidence suggest that another two essential conserved nuclear export factors Dbp5 and Gle1 play a major role in mRNP remodeling in the cytoplasmic side of the NPC. (118-119). Another line of evidence of a directional release of mRNP is exemplified by the Dbp5-ATP to Dbp5-ADP switch which induces the removal of specific protein factors including Mex67 and the poly(A) binding protein Nab2 (120).

Now coming to the previously asked question whether the same core set of export factors are involved in transporting both typical mRNAs and mRNAs which export slowly. The export factors precisely involved in the export mechanism are highly conserved. Though studies of different model organisms have provided the inconclusive report of their precise involvement. For example,

Mex67 is essential for mRNA export in the budding yeast *S. Cerevisiae* (5), but not in the fission yeast *Schizosaccharomyces pombe* (6) ; Yra1 is essential for export in *S. Cerevisiae* (7), but not in *Drosophila* or *Caenorhabditis Elegans* (8,9). This investigation gives us a conclusive idea that the same set of transport factor is not working globally. Therefore, it is logical to suggest that the same set of transport factors may not be involved in transporting both typical mRNAs and special mRNAs. In this regard, it is hypothesized that the special mRNAs may be recognized by transport factor little after of their contemporary typical mRNAs. In this regard, it is also suggested that conformational changes in the cis-element of the transcript body may delay its export by allowing another set of transport factors.

CBC and its role in mRNA transport: CBC, the cap-binding complex binds to the newly synthesized transcript. RNA Pol II synthesizes nascent transcript with a 5' triphosphate on the first nucleotide to which 7 methyl guanosine is incorporated via a 5'-5' triphosphate bridge. (19,20).The cap-binding structure protects the transcripts from exoribonucleolytic degradation (21,22).CBC was first isolated from the nuclear extract of HeLa cell using the 7mG as it has an affinity for the CBC (19, 23,24). CBC is composed of 20 and 80 KD polypeptides, which were designated as Cbp20 (cap-binding protein 20) and Cbp80 respectively. It is also demonstrated that the Cbp20 monomer is unstable in the absence of Cbp80, both in mammals and yeast (19,25-28). Therefore it is logical to say that complex of the two proteins is essential for the binding of a cap (29,23). Furthermore, Cbp20p has a structural similarity to many RNA binding proteins and is firmly attached with Cbp80p in such a manner that leaves the RNA binding surface of Cbp20p exposed. The highly conserved RNA binding surface plays an important role to keep the cap in place (20,30-35). It has reported that slowly exported special kinds of mRNAs are stabilized by *CBC1* in *Saccharomyces cerevisiae*. (1,2). Furthermore, *CBC1* of budding yeast encodes a protein similar to the orthologous nuclear cap-binding protein, CBP 80 in animal cells (3,32,33,23). In *Saccharomyces cerevisiae*, CBP80 has been isolated using both genetic and biochemical approaches (29, 36,38). CBP80 is not an essential gene (29,38). However, the deletion of the CBP80 gene shows a severe growth defect in budding yeast. CBP20 is also not an essential gene. Strains carry the null allele of both CBP20 and CBP80 are also viable (25,29).

Possibility of involvement of *CBC1* in transporting mRNAs especially special mRNAs cannot be ignored until the details mechanism of transport of special mRNAs is vividly explained. Export competent mRNP assembled with the nascent transcript at the very beginning of the transcription. As the transcription elongation proceeds the nascent mRNAs are bound by several factors. Many of the factors belong to the family of heterogeneous nuclear ribonucleoprotein (hnRNPs). hnRNP plays a pivotal role in various processes like packaging, export, and translation of mRNA. There are ten hnRNPs are reported in *Saccharomyces cerevisiae* (4,39). It is further demonstrated that in many steps of the mRNA transport pathway different hnRNP proteins interact with the mRNP.(4,39). The function of several hnRNP has been uncovered in budding yeast using genetic approaches. One of the extensively studied hnRNP proteins is Npl3p.It is already established that Npl3p acting as a carrier for mRNA transport from the nucleus (29,40,41). Several studies also suggest that Npl3 shuttles between the nucleus and cytoplasm. (81-85).Furthermore, it is found that shuttling of the RNA binding protein, Npl3, completely dependent on RNA synthesis. (86-88).Elegant studies of Shen et al., reveals that CBP80 and CBP20 interact with *NPL3* genetically. They further demonstrated, *NPL3* physically interacts with CBP80 and CBP20 in an RNA dependent manner. Coexisting *CBC1* with *NPL3* and RNA may further strengthen the idea of the possibility of a stimulatory role of *CBC1* in mRNA transportation. Stabilization of special mRNAs in *CBC1* deleted strain may indicate the possible involvement of *CBC1* in transporting the special mRNAs.

Role of *CBC1* and *RRP6* in mRNA degradation in the nucleus: Like cytoplasmic degradation of mRNA nucleolytic mRNA degradation has also extensively been studied. The nucleolytic degradation of mRNAs is well regulated to prevent degradation of all kinds of mRNA within the nucleus. Specific defects make an mRNA susceptible to the nuclear mRNA degradation machinery. Nuclear mRNA degradation is mostly exonucleolytic which allows mRNA to escape from nuclear degradation just protecting their ends. 5' ends of the nascent transcript are protected by the addition of a 7mGpppG cap structure and further by the addition of many other nuclear proteins. 3' poly-A tail is also protected by the sequential addition of several nuclear proteins (42).

Several studies revealed that Rrp6p found mostly in the nucleus (43-46). It is also demonstrated that the purified Rrp6p show 3'-5' exoribonucleolytic mode of hydrolysis (48). Error in mRNA processing and mRNP assembly leads to aberrant mRNA which may impair mRNA metabolism. To prevent the harmful effects of aberrant mRNA eukaryotic cells evolved some quality control mechanisms both in the nucleus and cytoplasm. (43,47). Quality control (QC) mechanism recruits some protein factor which in turn recruits some specific ribonuclease which degrades the aberrant mRNAs. In *Saccharomyces cerevisiae*, the nuclear ribonuclease exosome is made up of at least ten subunits of which mostly show 3' to 5' exoribonuclease activity. (49-51) . Several studies revealed that *RRP6* an important exosomal component exclusively localizes to the nucleus (48, 51-52). Furthermore, the strain mutant for the Rrp6p does not show any cytoplasmic mRNA turnover (53). Interestingly deletion of both *CBC1* and *RRP6* gene suppresses the rapid degradation of mRNA in the nucleus (54). It may be tempting to think about the actual mechanism by which *CBC1* and *RRP6* gene degrades mRNAs within the nucleus. It is already demonstrated that *CBC1* interacts with some nuclear export factor and by an unknown mechanism aids in mRNA transportation. Furthermore, it is demonstrated that preferentially retained special mRNAs within the nucleus are also degraded by the *CBC1* and *RRP6*. All of these studies lead us to conclude that *CBC1* and *RRP6* play a role in degrading nuclear mRNAs. Also, it will be of great interest to investigate whether any other factors or conditions function simultaneously with the *CBC1* and *RRP6* in degrading mRNAs preferentially retained within the nucleus.

Scanning cis-acting element: Recently Das et al., identified a stretch of nucleotides of about 202 in length in the transcript body as regulatory factor thought to responsible in retaining the special mRNA, *SKS1*, within the nucleus in budding yeast (2). It is already reported in the mammalian system that the signal sequence coding region (SSCR) acts as a nuclear export signal of an mRNA lacking an intron or functional cap (17). The systematic deletion of the *SKS1* transcript body revealed the regulatory element. Deletion mutant without having a cis-regulatory element would not show any propensity in retaining within the nucleus (2). To make their claim more strong Das et al., attached the above cis-acting element with the typical mRNA and showed that the chimeric mRNA retaining within the nucleus. However, their study did not uncover the mechanistic details of nuclear retention of the special mRNA, *SKS1*. Moreover, the 202 nucleotides long nuclear zip code may not be solely responsible as a retarding element. Their study also did not reveal the three-dimensional nature of the zip code sequences. Predicting the three-dimensional structure of the zip code sequence may reveal a more clear understanding of the nuclear retention of the special mRNAs.The possibility of more than one cis-element within the transcript body can not be ignored. Scanning the cis-acting element within special mRNAs other than *SKS1* is very important to stop the further argument that the cis-regulatory element is responsible for their slow export. And it was the important drawback of Das et al, not to include mRNA other than *SKS1* for cis scanning.

Osmostress and slower export of mRNAs: Special mRNAs get significantly stabilized in Cbc1 mutant strain as the transcript body smoothly exported out of the nucleus.Any unnatural phenomenon happening within the

living cell may indicate some possibility of urgent need within the cell. Das et al. got significant stability of the special mRNA *SKS1* without having cis-element in the cbc1 mutant strain (2). Though special mRNAs do not possess any processing defects still their retention within the nucleus and getting significantly stabilized in cbc1 deleted mutant strain arises a question in mind regarding the possibility of the *CBC1* gene in controlling slower export of the special mRNAs. It is well established that Cbc1 deletion is sensitive to osmotic stress and during osmotic stress translation of some gene in the cytoplasm gets perturbed in yeast (11). And obviously, transcripts of these genes will not be exported out of the nucleus on an urgent basis. It has been reported that *SKS1*mRNA encodes a serine/threonine-protein kinase required for the adaptation of the yeast cell in low glucose medium(5,16). Elena et al, further reported osmostress-responsive mRNAs are transcriptionally induced after osmotic stress in Cbc1 mutant yeast cell (11). The next question whether a mutation in the *CBC1* gene induces any kind of stress which may insist the special mRNAs to retain within the nucleus. Another interesting possibility of involvement of *CBC1* gene in mRNA export can not be ignored. In the HeLa cell line, it is already established that the interaction of CBC20 protein with RNA export factor is essential for the promotion of intronless mRNA export (18). So a further study is required to verify whether the special mRNA shows any link with osmostress-responsive mRNAs or not. Multitasking activities of *CBC1* are really convincing and demands extensive further studies. It will be of great interest to check whether the deletion of the *CBC1* gene impairs some unknown factor(s) solely responsible for smooth transport of intronless mRNAs, here *SKS1*, from the nucleus to the cytoplasm.

Kinetic competition between typical mRNA and special mRNA during mRNA transport :

After the synthesis by RNA Pol II, the nascent mRNA transcripts face the maturation phase and are loaded with messenger ribonucleoprotein particles (mRNPs) to make them competent for export from the site of transcription in the nucleus to the cytoplasm through the nuclear pore complex. It is suggested that mRNA makes its journey through the nucleoplasm by a diffusion-based mechanism. (68). Furthermore, several studies revealed that mRNAs traverse through nucleoplasm not being driven by any energy-dependent motor apparatus rather its travel by random Brownian motion (69-78). Several lines of evidence confirmed that during the transportation, mRNA interacts with several protein factors to make their journey successful from the nucleus to the cytoplasm (66). Before reaching the cytoplasm every mRNA has to overcome the hurdle of crossing the nuclear pore complex (NPC). It is suggested that a G1 cell nucleus of yeast contains about 90 NPCs (67). It is well established that the nuclear pore complex itself plays a major role in gene regulation by monitoring the export of mRNA (69). The next key question is how the nuclear abundance of mRNA influences the movement of an mRNA. How many transcripts are producing at a particular time? Whether inter species mRNA interaction exists or not. By measuring the decay rate and half-life mRNA abundance can be quantified. Most of the cases the decay rate or half-life of mRNAs are measured by blocking the transcription machinery after the addition of RNA polymerase inhibitor or using RNA polymerase II thermosensitive mutants (56,57,58). We are not able to measure the mRNA abundance naturally without perturbing cellular activities. Naturally, an experimental result does not reflect the absolute scenario happenings within the cell. Furthermore, it is suggested that artificial perturbation in any physiological process like transcription develops an unusual condition that imparts unusual gene expression or makes different in mRNA degradation mechanism. (56,59) . Both the rate of synthesis and decay determines the abundance of mRNAs. The steady-state level achieves when the rate of synthesis and degradation are equal. (56). So, synthesis is a zero-order reaction. The abundance of nuclear mRNA has a direct link with the transport mechanism. In the quality control circuit of RNA, there is a kinetic competition that exists between the processes of new RNA synthesis and the

degradation of the targeted RNA. (47). In the context of nuclear export, kinetically incompetent mRNAs may have the tendency to retain within the nucleus and could be susceptible to the nuclear degradation machinery (1,2). In this regard, it is logical to suggest that there should be a kinetic competition between the normally exported mRNA and the slowly exported mRNAs that preferentially retained within nucleus. mRNAs are also competitive to get access to the vicinity of the NPC during transportation. We do not know how many mRNAs are running. We do not know whether mRNA has the specificity for a particular NPC. We also do not know an mRNA approaches to how many of NPC for a successful journey to the cytoplasm. To date, we have also no idea how much time an mRNA takes to reach the NPC. Due to many reasons, several mRNAs will be defeated in the competition for reaching up to the NPC. RNA length may be considered as one of the key factors for successful transportation through NPC (79-80). It is reported that longer mRNAs are more successful in transporting them through the NPC (121). Microarray analysis has revealed that several normal mRNAs are retained within the nucleus preferentially and susceptible to nuclear degradation machinery.Further it is revealed that these normal special mRNAs are exported out of the nucleus slowly (1). But it is not clear to us how slow they are? Here time is an important factor to understand the rate of slow export. Several studies revealed that RNA polymerase II traveled 18-42 nucleotides per second on the Chromosome template (60-64). It takes about 25-50 seconds for the 1kb yeast gene (65). Halting the speed delays the release of mRNA from the chromosomal template (63).

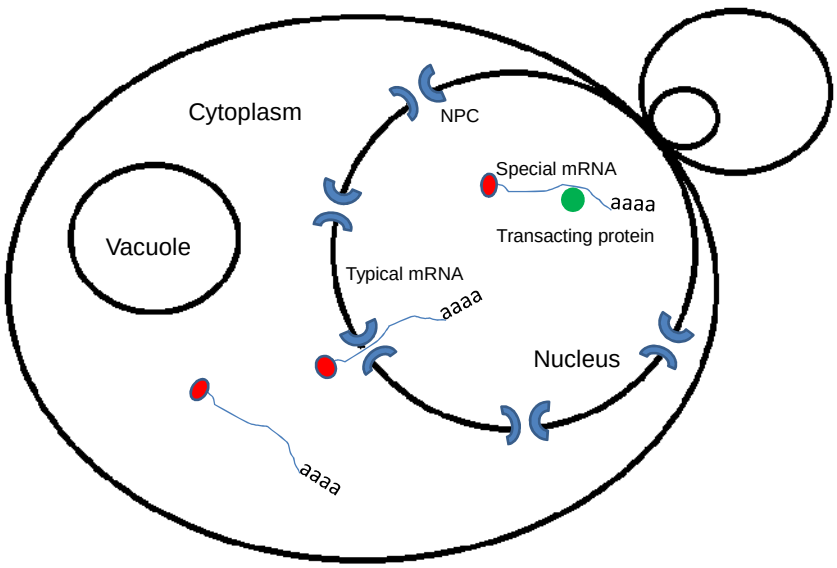

Fig. 1. Competition between special mRNA and typical mRNA during nucleo-cytoplasmic transport in budding yeast, *Saccharomyces cerevisiae*

A competition is existed in between special mRNAs (*SKS1*, *IMP3* & *YLR194C*) and typical normal mRNAs (*ACT1* & *CYH2*) in respect of mRNA transport. It is assumed that mRNA synthesis is a zero-order reaction and mRNA decay or degradation is a single order reaction. During the steady-state kinetics of an mRNA, the rate of synthesis and the rate of decay will be the same.

Exploring the trans-acting factor responsible for slow export: To date, it is not clear to us how the normally exported mRNAs are distinguished from the special mRNAs exported slowly. Also, it will be a great interest to investigate what are the minimum factors and interactions essential for the preferential nuclear retention of the special mRNAs. Numerous studies confirmed the existence of several proteins responsible for retaining the aberrant mRNAs within the nucleus (90,91,92). However, no complete ideas have been suggested to uncover the underlying mechanism of nuclear retention of the special mRNAs. It can be suggested that the cooperative binding of RNA binding proteins (RBP) assists the nucleoplasmic Brownian movement of mRNAs. In this regard cooperative interactions of RBP with the export receptor and nuclear basket associated protein may also be suggested. It is revealed that mRNAs get earmarked during transcription for the nucleo-cytoplasmic transportation (91). Therefore it is logical to suggest that mRNAs that exported slowly had already been marked co-transcriptionally for slow movement. It may be tempting to think about the real situations the special mRNAs face. Is only the cis-acting RNA element responsible for the slower export? Definitely, there should be some trans-acting protein factors supposed to interact with the cis-acting mRNA element resulting in slower export of the special mRNAs. The next key question is when the transacting protein factor binds to the corresponding mRNA element and when it detaches from the mRNA? A clue to this question may come by hypothesizing a model which deals with the switch off and switch on state of RBPs bound to mRNAs. In switch on mode phosphorylated or hyper-phosphorylated condition of RBP could change its conformation thereby RBP interacts with a defined set of export factors with more affinity and aids in the successful transportation of mRNAs. On the other hand in switch-off mode de-phosphorylated or hypo-phosphorylated RBP stays in its native state resulting limited interactions or no interaction with an export factor or nuclear basket protein and fails to nucleo-cytoplasmic transportation of aberrant mRNAs as well as mRNAs exported slowly. Therefore, this model indicates that less affinity of RBP to the nuclear receptor and nuclear basket protein makes the special mRNAs partially export incompetent. Interestingly, like aberrant mRNAs, slowly exported special mRNAs do not completely retain within the nucleus and degraded by the nuclear degradation machinery. The limited interaction of special mRNAs with the export factors and nuclear basket protein facilitates them not to retain completely within the nucleus.

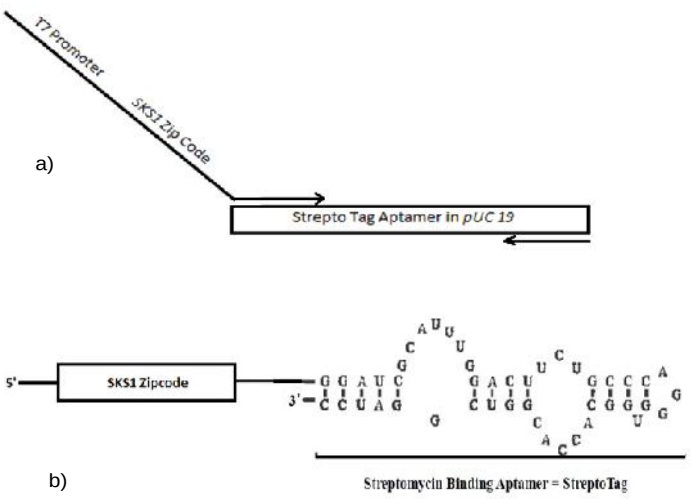

Fig. 2.Construction of RNA aptamer: (a) & (b).Preparation of RNA aptamer using PCR where forward primer includes the T7 promoter and the backward primer includes the whole Strepto Tag Sequence.

Normal mRNAs are generally exported out of the nucleus and take part in the protein synthesis machinery. However, a certain class of mRNAs takes part in protein synthesis machinery only after reaching their final destination. These mRNAs are marked for the localization. The next key question is how they are marked? Initially, the localization of the mRNAs is determined by the presence of a cis-acting element within the transcript body (89). It is suggested that a 202 nucleotide (nt) long zip-code, identified within the transcript body of the special mRNA, *SKS1*, is responsible for the preferential nuclear retention (2). An important question concerns how the cis-acting RNA element aids preferential nuclear retention? In this regard, it is further suggested that the zip-code element that presents within the transcript body would be recognized by a number of transport complexes (90). Several studies revealed that a series of signal situated within the 3' UTR that influences the binding of trans-acting factors to the RNA (91,92). After binding, the protein factors may change the folding nature of the RNA that ensures the further cooperative binding of the other factors resulting smooth journey of the RNA. It is already suggested that putative cis-acting zip code is responsible for the slower export of the special mRNAs (2) However several questions, regarding the involvement of trans-acting protein factors in transportation of special mRNAs remain to be answered. Also, it will be of great interest to investigate the factors believe to have interacted with the cis-acting elements present within the transcript body of special mRNAs.

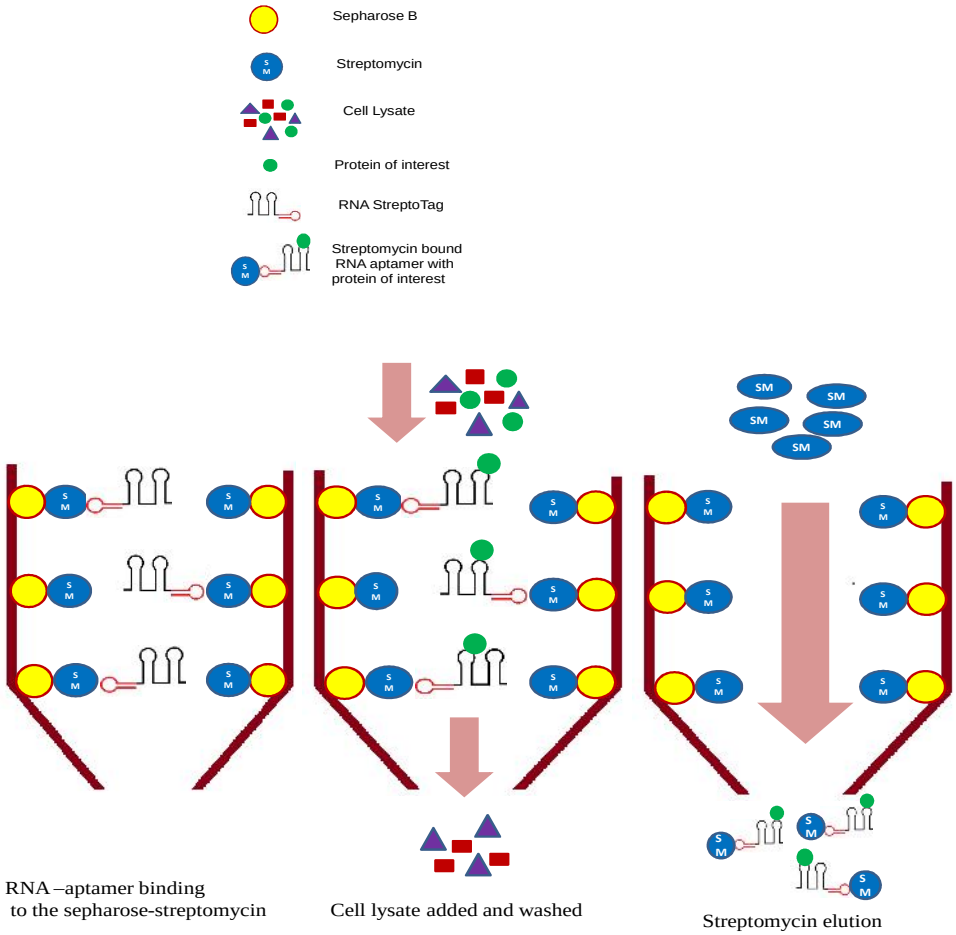

Fig.2.C. The Strepto Tag procedure: Zip code sequence tagged to the streptoTag (in red) is bound to streptomycin (SM) immobilized to the Sepharose. After application of total cell lysate (colored shapes) and washing RNA-protein complexes with specifically binding protein (green circle) can be eluted by the addition of free streptomycin.

Trans-acting protein factors play a pivotal role as an RNA-binding protein and facilitate localization and anchoring of mRNA in distinct sub-compartment of the cell (93-97). Discovering a new RNA-binding fold further strengthens the idea of RNA-protein complexes in the cell (98). For further characterization and identification of the potential zip code binding-partners of the special mRNA, an efficient purification technique is required. In this regard streptoTag, a simple one-step affinity purification method may be suggested. The strepto Tag method permits easy purification of high- and low-affinity RNA binding proteins. In this method, StreptoTag, the streptomycin –binding aptamer is fused to either end of the mRNA of interest (99). RNA aptamers are single-stranded RNA oligonucleotide with unique tertiary structure binds to the target with high affinity and specificity (100). Here zip code sequence found in the special mRNA is to be fused with an RNA aptamer using the in-vitro transcription. The prepared RNA hybrid that consists of an aptamer sequence with high binding specificity to the aminoglycosidic antibiotic streptomycin with a dissociation constant (K_D) of around 1µM and putative zip code sequence is incubated with the total cellular lysate (101). Now the complex mixture is applied to an affinity column containing streptomycin immobilized to sepharose. The RNA aptamer will facilitate the binding of invitro-assembled RNA-protein or RNA-RNA complex to streptomycin-sepharose. Subsequent elution with free streptomycin facilitates the efficient recovery of specific ribonucleoprotein or RNA-RNA complexes (93,101).

Conclusion

Genetic information flows via nucleocytoplasmic translocation of mRNAs. During the nucleocytoplasmic translocation, a particular mRNA faces many events. Monitoring the eventful journey of a particular mRNA from the nucleus to cytoplasm still now beyond our capacity. It is well accepted that the nucleocytoplasmic movement of mRNAs is Brownian and not driven by a motor (69). We are already seen that few special kind mRNAs show slower movement than that of the typical mRNAs. The understanding of the slower nature of mRNA movement is a matter of future investigation. Das et al tried to figure out why the special mRNAs preferentially retain within the nucleus. They concluded that a Zipcode sequence is responsible for the slower export of a particular special mRNA, SKS1(2). A single-molecule experiment in metazoan cells revealed that mRNAs transported in a discontinuous manner where a period of fast diffusion is intermitted by periods of much slower mRNA movement (69). It is thought that the slower movement of mRNAs due to the molecular crowding effect. The cell cytoplasm is a multicomponent solution where cosolutes can influence the bimolecular folding of RNAs due to molecular-crowding effects (122). It is found that diffusion of mRNA slows down when the mRNA enters a chromatin-dense region. (69). It is demonstrated that macromolecular crowding can reduce diffusion rates and enhance the binding rates of the macromolecule. (123,124) Zipcode sequence may be one of the reasons for the slower export of the special mRNA. But analysis of other special mRNAs other than SKS1 is required to strengthen the zip code theory of slower export. Considering mRNA movement as Brownian it is calculated that the transit time of an mRNP across a typical mammalian cell nucleus is in the range of 2-6 minutes. But many nuclei are not perfect spheres rather it is ellipsoidal. The ellipsoidal nucleus reduces the distance an mRNA has to travel to reach the periphery in one dimension (69). Finally, it depends on where a gene is located within the nucleus. May be special mRNA, SKS1is located distally than that of the typical mRNAs resulting in much retention time of the SKS1 mRNA within the nucleus and has to face the nuclear degradation machinery. Moreover, it is thought that the retention factor, zip code sequence of the special mRNAs will interact with many other proteins during the nucleocytoplasmic journey. In this regard, elaborate studies of the transacting protein factor are essential to know the true nature of the slower export of the special mRNAs. Using the StreptoTag affinity purification technique the transacting protein (s) can easily be purified. So, further biochemical approaches are required to

explore the slower movement of the special mRNAs in detail.

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