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Running Head: Transdifferentiation by ribosome

Abstract

Ribosomes are intracellular organelles ubiquitous in all organisms, which translate information from mRNAs to synthesize proteins. They are complex macromolecules composed of dozens of proteins and ribosomal RNAs. Other than translation, some ribosomal proteins also have side-jobs called “Moonlighting” function. Majority of these moonlighting functions influence cancer progression, early development and differentiation. Recently, we discovered that ribosome is involved in the regulation of cellular transdifferentiation of human dermal fibroblasts (HDFs). *In vitro* incorporation of ribosomes into HDFs arrests cell proliferation and induces the formation of cell clusters, that differentiate into three germ layer derived cells upon induction by differentiation mediums. The discovery of ribosome induced transdifferentiation, that is not based on genetic modification, find new possibilities for the treatment of cancer and congenital diseases, as well as to understand early development and cellular lineage differentiation.

Introduction

Ribosomes are intracellular organelles present in all living organisms. Although there are minor structural differences between eukaryotic and prokaryotic ribosomes, in general ribosomes are composed of approximately 50 to 100 ribosomal proteins and several ribosomal RNAs (Wilson and Doudna Cate, 2012). The most known function of ribosome is translation that is decoding mRNA sequence into protein depending on the codon. However, some ribosomal proteins are also involved in other biological processes, like development, cellular differentiation and cancer progression, which are called “Moonlighting” activity (Jeffery, 2003).

Previously, we found that lactic acid bacteria (LAB) could induce cellular transdifferentiation in human dermal fibroblasts (HDFs) (Ohta et al., 2012). Another group later reported that Leprosy bacterium, *Mycobacterium leprae* can efficiently expand their habitat by regulating the cellular differentiation system of the host (Masaki et al., 2013). More recently, the efficiency of artificial cellular reprogramming has been improved by bacterial proteins while they were forcibly expressed along with the Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) (Ikeda et al., 2017). These results indicate that bacterial intrinsic factors have the potential to direct cellular reprogramming and transdifferentiation (Ito and Ohta, 2015).

Recently, we confirmed that ribosomes are the actual component for the induction of LAB

mediated cellular transdifferentiation (Ito et al., 2018). Trypsin digested HDFs form cell clusters upon treatment with ribosomes from both eukaryotic and prokaryotic sources (Fig. 1). Although the biochemical properties of these cell clusters vary from those of pluripotent stem cells, they clearly possess the multipotency since they differentiate into different germ layer derived cells upon induction with respective media.

In this review, first we compare the common points about the structure and function of prokaryotic and eukaryotic ribosomes. Then, we discuss the moonlighting functions of some ribosomal proteins as well as some alternative regulatory approaches taken by the ribosome itself. Our main focus is the induction of cellular transdifferentiation by ribosome and its possible implications for better understanding of early development and cellular differentiation.

A molecular view of ribosomal structure

From the evolutionary point of view ribosome can be categorized into two types: the bacterial ribosome 70S represents the prokaryotic type and for the rest of the species 80S represents the eukaryotic type (Spahn et al., 2001). Each type consists of one small subunit (SSU) and one large subunit (LSU). 30S SSU and 50S LSU are the comprising subunits of 70S prokaryotic ribosome. 30S SSU has one ribosomal RNA (rRNA) namely 16S (1540 nucleotides) along with 21 proteins. On the other hand, 50S LSU contains 5S (120 nucleotides) and 23S rRNAs (2900 nucleotides) with 34 proteins. The eukaryotic 80S ribosome is comprised of 40S SSU and 60S LSU. 40S SSU is further divided into one 18S rRNA (1753 nucleotides) plus 33 proteins. And 60S LSU is divided into 28S (3354 nucleotides, in human) or 25S (3363 nucleotides, in yeast), 5.8S (154 nucleotides) and 5S (120 nucleotides) rRNAs along with 47 protein moieties (Filipovska and Rackham, 2013). Two special kinds of eukaryotic ribosome have been observed in mitochondria and chloroplast, which are originated from prokaryotic ancestors (Bieri et al., 2017; Wilson and Doudna Cate, 2012). Mitochondrial ribosome is also known as mitoribosome. In yeast the 74S mitoribosome is consisted of 37S SSU and 54S LSU (Amunts et al., 2014). The chloro-ribosome shares homology with prokaryotic 70S ribosome with few differences. In case of spinach (*Spinacia oleracea*), 50S LSU consists of an additional 4.8S rRNA along with the prokaryotic 5S and 23S rRNAs, and two plastid specific ribosomal proteins – PSRP5 and 6 (Ahmed et al., 2016). A type of translational machinery similar to ribosome has also been reported recently in virus. Having no ribosome alike machinery, Tupanviruses, a member of mimiviridae found in amoebae, can process tRNA/mRNA

maturation, protein modification as well as all translational steps through upto 70tRNA, 20aaRS and 11 factors (Abrahão et al., 2018).

By the robust advancement of X-ray crystallography and Cryo-Electron Microscopy (Cryo-EM) more clear and complex molecular structure of ribosome at higher resolution has been observed in recent years. Eukaryotic and prokaryotic SSU share common structural hallmarks, that is, SSU could be subdivided into head, platform, body, left foot, right foot, shoulder and beak (Frauenfeld et al., 2011; Matzov et al., 2017). Most of ribosomal proteins are conserved in both eukaryotes and prokaryotes indicating the similar patterns of activities as a protein manufacturing machinery. From the results of Cryo-EM observation of ribosomes from yeast and wheat germ, the position of eukaryotic ribosomal proteins was clarified (Armache et al., 2010). The eukaryotic ribosomes are composed of 80 proteins sharing the common achaea-eukaryotic spatial positions within the ribosomal subunits. These positions are distinguished from the prokaryotic counterparts by variable and expansion segments. Like neural-synaptic communication, ribosomal proteins develop regulatory circuit to flow the information of protein synthesis by interconnecting these variable and expansion segments (Poirot and Timsit, 2016). Both in prokaryotes and eukaryotes, the multifaceted structural organizations of ribosomal subunits along with the protein moieties explain the dynamic translational processes within all living organisms.

Moonlighting of ribosomal proteins

There are several instances where a single protein is found to perform multiple functions on different cellular locations or in response to different environmental cues, without making a change to its primary structure. These proteins are called moonlighting proteins and many previously known proteins are joining this class defying the classical one gene – one protein – one function view (Gancedo et al., 2016; Jeffery, 2014, 1999; Wang et al., 2014). Housekeeping proteins like phosphoglucose isomerase, glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase M2 have also been reported to carry diverse functions as well as their enzymatic role in energy metabolism (Jeffery, 2016; Sirover, 1999). Ribosomal proteins also possess moonlighting functions in addition to their conventional roles as part of the protein synthesizing machine. Individual ribosomal proteins have been reported to perform different biological functions, while the majority of these proteins are engaged in tumorigenesis, immune signaling and development (Warner and McIntosh, 2009; Zhou et al., 2015).

Cancer cells require higher number of ribosomes compared to normal cells, to meet the increased demand of protein synthesis to maintain their rapid proliferation. Remarkably, some individual ribosomal proteins as well as the whole ribosome are also found to be associated with cancer progression – presenting both oncogenic and tumor suppressing functions. Such functions are mostly mediated by the interactions of individual ribosomal proteins with various cellular processes like apoptosis, cell cycle regulation or cell migration. Mechanistically, majority of these interactions rely on the tumor suppressor protein p53, while others utilize c-Myc, NF-κB etc. (Xu et al., 2016). Zhou et al. have listed more than 40 ribosomal proteins bearing moonlighting functions and classified them according to their functional involvements (Zhou et al., 2015). In this review, we will mention only a handful of those functions.

RPS6 and phospho-RPS6 both are overexpressed in non-small cell lung cancer, and *in vitro* depletion of RPS6 inhibits the cancer cell proliferation by inducing G0-G1 cell cycle arrest (Chen et al., 2014). RPL5, RPL11 and RPL23 function as tumor suppressors by inhibiting MDM2 mediated ubiquitination of p53 and thereby augmenting p53 activity (Dai and Lu, 2004; Jin et al., 2004; Zhang et al., 2003). RPL26 is another tumor suppressing ribosomal protein which has the dual ability of regulating MDM2 as well as enhancing p53 mRNA translation (Takagi et al., 2005). Apart from these p53 utilizing ribosomal proteins, some tumor suppressing ribosomal proteins also function by inactivating the oncoprotein c-Myc. RPL11 is one such protein which downregulate c-Myc function both by inhibiting its transcription and directing mRNA degradation (Challagundla et al., 2011; Dai et al., 2007). RPL13 is a major anti-inflammatory protein which functions by forming the IFN-γ activated inhibitor of translation (GAIT) complex (Mazumder et al., 2003; Mukhopadhyay et al., 2009). Some ribosomal proteins are also reported to be associated with developmental disorders, commonly called ribosomopathies. Diamond-Blackfan Anemia (DBA), which impairs the bone marrow function is an example of such disease. Initially, mutation of RPS19 was found to be responsible for DBA, but later a number of other ribosomal proteins were also reported to be mutated in DBA patients (Doherty et al., 2010; Draptchinskaia et al., 1999; Gazda et al., 2008). DBA patients also show a reduction of the intracellular ribosome amount which is responsible for the altered hematopoietic stem cell differentiation (Khajuria et al., 2018). Recent studies in rice also showed that, expressions of several ribosomal proteins are changed in response to biotic and abiotic stresses (Moin et al., 2016; Saha et al., 2017).

The classical view considers ribosome as an obedient worker which synthesizes protein as

directed by the genome. This view is now being seriously challenged by various discoveries on different ribosomal proteins, their differential expression patterns and their correlation with different biological events. Ribosome filter hypothesis was proposed in 2002 by Mauro and Edelman when they attributed regulatory role to ribosomal subunits suggesting that they can preferentially bind particular mRNAs and thus favor their translation over others (Mauro and Edelman, 2007). Ribosomal protein related diseases where only specific tissues are affected raises questions about the uniformity of ribosomal constituents across all tissues. Model eukaryotic organisms, *Saccharomyces cerevisiae* and *Arabidopsis* genomes contain multiple copies for each ribosomal protein, and several paralog specific events have been found in both organisms where only one of the multiple copies of a single ribosomal protein was found to be indispensable (Carroll and Wickner, 1995; R. F. Degenhardt and Bonham-Smith, 2008; Rory F. Degenhardt and Bonham-Smith, 2008; Ohtake and Wickner, 1995). From these observations, ribosome has been viewed as a cloud of all the possible ribosomal proteins and the functional ribosome in each tissue would specifically combine the required set of ribosomal proteins to form 'specialized ribosome' (Dinman, 2016). Indeed, a recent report has confirmed the existence of heterogeneous ribosomes in the mouse embryonic stem cells, as well as the preferential translation of particular mRNAs by these ribosomes (Shi et al., 2017). Counting all these evidences, we can say that the ribosome still remains a mysterious macromolecule and we are yet to fully appreciate its influence on the living system.

Cellular reprogramming by ribosome

Trypsin digested HDF cells were clustered by using His-tagged ribosomes (Ito et al., 2018) (Fig. 1). The incorporation of external ribosomes inside the cytosol and nucleus of clustered cells was confirmed by His-tag immunostaining. Ribosome treatment of non-trypsin digested HDFs shows neither cell cluster formation nor uptake of ribosomes. The diameter of whole ribosome is about 20 nm (Stark et al., 1995), which is huge considering a protein complex. But endosomal vesicles are about 10 μm in size, which is sufficient to incorporate ribosomes and even bacteria (Kaksonen and Roux, 2018; Shamir et al., 2016; Veiga et al., 2007). Since trypsin digestion activates cellular endocytosis and cell cluster formation was inhibited by endocytosis inhibitors (Serdiuk et al., 2014; Ito et al., 2018), incorporation of ribosomes is considered to proceed through endocytosis. Ribosome incorporated cell clusters (RICs) also differentiated into three germ layer derived cells upon induction with specific media *in vitro*, namely ectodermal neurons, mesodermal cardinomyocytes and endodermal hepatocytes. A proposed model for RICs formation is shown in

Fig. 2.

It is interesting to note that ribosomes of all different origins tested in our experiment possess the cell cluster inducing ability, irrespective of their phylogenetic or structural differences. Since the exact mechanism behind RICs formation is still unknown, we looked up the commonality among these ribosomes. There have been multiple reports of functional hybrid ribosomes prepared in laboratories, which indicate at least some degree of complementarity among ribosomes from different biological origins. GTPase, a part of ribosome activity for translocation of amino acids, can be replaced between eukaryote, prokaryote and archaea (Uchiumi et al., 1999, 2002). The antibiotic binding site on the 16S rRNA of bacterial ribosomes can be replaced by the complementary RNA sequences from eukaryotes, resulting bacterial hybrid ribosomes which are both functional and antibiotic resistant (Hobbie et al., 2007). However, the common function of all these ribosomes – translational activity has no part in the induction of transdifferentiation. Addition of gentamicin, which inhibits prokaryotic translation by binding to the small subunit of prokaryotic ribosomes, does not inhibit cell cluster formation. Moreover, in the experimental condition it is expected that the subunit structure of the exogenous ribosomes is dissociated. Maintenance of the whole ribosome structure requires 15 mM Magnesium (Nierhaus, 2014). But cell clusters do not form in presence of such high concentrations of Magnesium (unpublished data). Therefore, we removed Magnesium from cell culture medium before assay of the cell cluster formation. The above discussion leaves us with the possibility that individual ribosomal protein(s) is/are responsible for triggering the transdifferentiation.

RICs exhibit some physiological and biochemical differences from known pluripotent stem cells (Ito et al., 2018). One obvious difference is the cessation of cell division in RICs. Upon ribosome treatment, the cluster formation proceeds by the aggregation of surrounding cells, not by cellular proliferation. Once the clusters are formed, cell division is not observed during 14 days of culture. According to definition, stem cells not only produce differentiated daughter cells, but they are able to self-replicate as well. Immunocytochemical staining and single cell quantitative PCR analysis showed that not all cells within RICs express the pluripotency markers OCT3/4 and NANOG. RICs also failed in teratoma formation or chimeric mouse generation. Functional assessment of pluripotent stem cells classify these cells according to their differentiation capacity, autonomous replication ability, tumor forming ability and chimera forming ability (De Los Angeles et al., 2015). Considering these hallmarks, RICs are not classified as stem cells.

Conclusion

Considering the abundance of various microbes in the gastrointestinal tract and the ability of ribosomes to induce transdifferentiation, the intestinal microenvironment is a huge area of interest. The endocytosis facilitating enzyme, trypsin is also available in the same environment. It will be interesting to see the interaction between the cells and exogenous ribosomes in the physiological setting. Since activation of transdifferentiation requires incorporation of a large number of ribosomes, induction of transdifferentiation *in vivo* may be low potency in general. However, in the long way of evolution, there is a rare chance of exposure to high-density ribosomes by damage, inflammation or bacterial infection. Elucidation of the exact molecular mechanism of RICs formation and the molecular roadmap of RICs progression is important to understand its influence in the living body. Our discussion speculates that one or a few of the ribosomal proteins might be responsible for triggering the transdifferentiation. The cessation of cell division upon ribosome treatment also raises questions which are yet to be answered. The prime question that strikes us is – what might happen if cancer cells are treated with ribosomes. We hope that resolving these unanswered questions will facilitate better understanding of cellular development and open up new avenues of therapeutic interventions.

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Conflicts of interest

All authors declare that no potential conflicts of interest exist.

Figure legends

Fig. 1. (A) Induction of the ribosome incorporated cell clusters (RICs). *E. coli* JE28 encodes His-tag modified ribosomal protein which enables purification of ribosomes by affinity chromatography (Ederth et al., 2009). Experimental scale is applicable for 24 well plate (Thermofisher No.142475) and/or 4 well plate (Thermofisher, No. 176740). Cell culture medium SCM130 (PluriSTEM™ Human ES/iPS Medium) was purchased from Merck Millipore. (B) RICs, 14 days after ribosome incorporations. Bar = 100 μm.

Fig. 2. Proposed model of cellular transdifferentiation by ribosome. Bacteria and ribosomes both are incorporated into host cells by trypsin activated endocytosis. Bacteria remain in cytosol, but ribosomes enter the nucleus and possibly trigger transdifferentiation. In case of LAB driven differentiation, LAB rupture inside cytosol and the released ribosomes enter nucleus to exert their effect.

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