

The ribonome: a dominant force in co-ordinating gene expression

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The ribonome is the total cellular complement of RNAs and their regulatory factors functioning dynamically in time and space within ribonucleoprotein complexes. We theorize that the ribonome is an ancient central co-ordinator that has evolved to communicate on multiple levels to the proteome on the one hand (feed-forward), and the transcriptome and RNA processing machinery on the other (feed-back). Furthermore, the ribonome can potentially communicate to other cells horizontally with implications for biological information transfer and for the evolution of both RNA and DNA operating systems. The post-transcriptional RNA operon theory of co-regulated gene expression accounts for the co-ordinated dynamics of RNA-binding proteins within the cellular ribonome, thus allowing for the recombination and remodelling of the RNPs (ribonucleoproteins) to generate new combinations of functionally related proteins. Thus, post-transcriptional RNA operons form the core of the ribonomic operating system in which both their control and co-ordination govern outcomes. Within the ribonome, RNA-binding proteins control one another's mRNAs to keep the global mRNA environment in balance. We argue that these post-transcriptional ribonomic systems provide an information management and distribution centre for evolutionary expansion of multicellularity in tissues, organs, organisms, and their communities.

The Ribonome

The ribonome operates at multiple levels of gene regulation to control and co-ordinate transcription, splicing, export, stability, and translation (Keene, 2007a; Halbeisen et al., 2008). It is a central regulatory environment that is both self-sustaining and self-limiting. In mammalian cells the ribonome consists of thousands of RBPs (RNA-binding proteins) and their associated mRNAs, non-coding regulatory RNAs and auxiliary proteins that are contained in mRNPs [messenger RNPs (ribonucleoproteins)]. The importance of the ribonome is highlighted by the fact that the steady-state levels of proteins within the proteome of a cell tend to have an imprecise correlation with the steady-state levels of mRNAs within the

transcriptome, suggesting that additional regulation must be occurring (reviewed in Keene, 2001; Keene and Tenenbaum, 2002). RBPs are found in all organisms and surprisingly outnumber DNA-binding factors almost two to one in eukaryotes with approx. 2500 or more proteins estimated to function as RNA interactors (Keene, 2001; Gerber et al., 2004). *Saccharomyces cerevisiae* has hundreds of RBPs but the same ribonomic organizational principles appear to operate (Gerber et al., 2004; Keene, 2007a; Halbeisen et al., 2008; Komili and Silver, 2008; Saint-Georges et al., 2008). Moreover, evidence for co-ordinately regulated post-transcriptional operons and decay regulons now exists for archaea (Andersson et al., 2006), and decay regulons have been demonstrated in bacteria (Bernstein et al., 2004; Halbeisen et al., 2008), supporting the ribonome's importance

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Key words: exosome particle, post-transcriptional operon, RNA regulon, RNA stability, translational regulation, USER code.

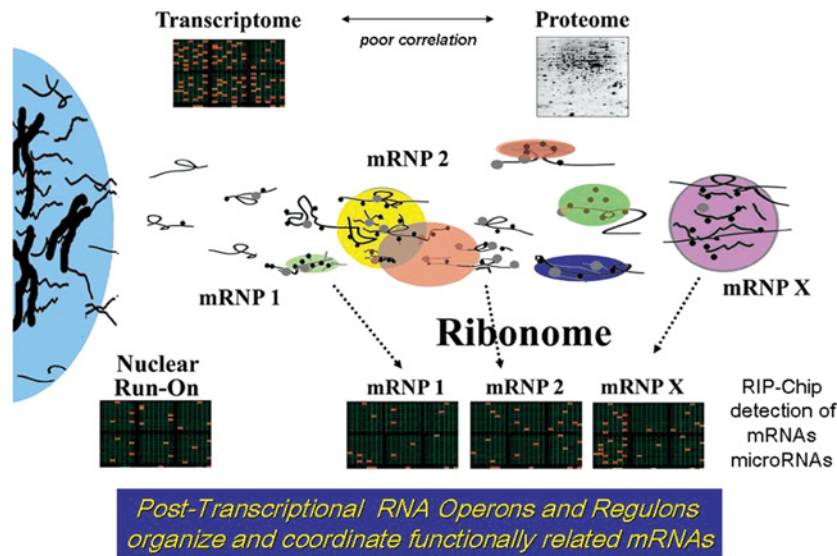
Abbreviations used: ARE, AU-rich element; BRF1, butyrate response factor 1; PTRO, post-transcriptional RNA operon; RBP, RNA-binding protein; RIP-Chip, RNP immunoprecipitation-microarray; RNP, ribonucleoprotein; hnRNP, heterogeneous nuclear RNP; mRNP, messenger RNP; SIRT, sirtuin (silent mating type information regulation 2 homologue); TIA-1, T-cell-restricted intracellular antigen-1; TIAR, TIA-1-related protein; TTR-RBP, turnover and translation regulatory RBP; USER, untranslated sequence element for regulation.

Ribonome: The cellular ribonucleoprotein environment containing mRNAs, non-coding regulatory RNAs and proteins. The ribonome is highly dynamic and responsive to cell signalling.

Messenger ribonucleoproteins (mRNPs): mRNPs consist of proteins that bind directly and proteins that associate indirectly with RNAs and the RNA components including the mRNAs and non-coding regulatory RNAs. mRNPs are generally very stable in content unless a cellular perturbation or chemical signal are imposed on the cell.

Figure 1 | Visualization of the analysis of RNPs within the ribonome

The organization and co-ordination of RNPs that drive post-transcriptional gene expression within the ribonome as experimentally determined using RIP-Chip to detect mRNA and microRNA components of RNP complexes. The finding that functionally related mRNAs are associated with specific RBPs within mRNPs led to the post-transcriptional RNA operon/regulon model of co-ordinated gene expression. Subsequently, other studies using polysome gradient profiling, RNA stability-microarrays and high throughput deep sequencing demonstrated that subsets of functionally related mRNAs can be co-ordinated at the levels of translation and stability (reviewed in Keene, 2007a). Reproduced from Keene (2001) with permission. © (2001) Proc. Natl. Acad. Sci. U.S.A.



in gene expression across all domains of the Tree of Life.

The theory of the ribonome and the PTRO (post-transcriptional RNA operon) is founded on the premise that discrete subsets of mRNAs are assembled and co-regulated in eukaryotic cells via a mRNP-driven process (Figure 1; Keene and Tenenbaum, 2002). RBPs and small non-coding RNAs are multi-targeted to functionally related mRNAs and act as *trans*-acting factors within the RNP infrastructure to co-ordinate mRNA expression via *cis*-acting elements contained within the mRNAs (Keene, 2001; Bartel, 2004; Jing et al., 2005; Elemento et al., 2007). Multiple *cis*-regulatory elements exist on each mRNA and result in

a unique, yet modular USER (untranslated sequence element for regulation) code (Keene and Tenenbaum, 2002). These USER codes therefore determine which RNA-binding factors can associate with each message in order to co-operate or compete, and ultimately regulate the fate of that mRNA. Multiple RNA-binding factors can associate with a single message simultaneously or sequentially and a single RBP or non-coding regulator RNA can potentially target a large number of different mRNA species (Gao et al., 1994; Tenenbaum et al., 2000; Bartel, 2004; Keene, 2007a; Komoli and Silver, 2008; Halbeisen et al., 2008; Hendrickson et al., 2008; Hogan et al., 2008; Landthaler et al., 2008). It is the co-operation and competition among these factors which results in

Post-transcriptional RNA operon/regulon (PTRO): The concept that functionally related groups of mRNAs are co-ordinately regulated by multi-targeted transacting factors in time and space. Many PTROs have been recently demonstrated that mediate co-ordinated mRNA decay, translational activation and RNA localization of multiple mRNAs that encode macromolecular subunits and components of pathways. Eukaryotic mRNAs are used combinatorially as members of distinct PTROs to allow the multifunctional proteins they encode to be co-ordinated in multiple cellular settings.

Untranslated sequence element for regulation (USER): Modular units of 3' or 5' untranslated regions of mRNA that embody a complete regulatory unit. USERS are not synonymous with the binding sites of RNA-binding proteins or small regulatory RNAs but consist of such elements in a functional configuration. USERS may occur in coding regions as well but they appear to be rare.

the final combinatorial codes of regulation of a given message. However, it is not simply a matter of controlling a single mRNA's fate, but rather the intricate harmonization of groups of messages that underlie the functioning of PTROs. For example, the stability of multiple mRNAs change together in a co-ordinated fashion following cell perturbations, whether internally or externally derived (Lam et al., 2001; Wang, Y. et al., 2002; Yang et al., 2003; Grigull et al., 2004; Raghavan et al., 2004; Cammas et al., 2008; Lukong et al., 2008; DeGracia et al., 2008). This co-ordinated orchestration of related mRNAs through turnover, localization and/or translatability provides the cell an agile means to rapidly adapt to its ever-changing environment.

Differences between the ribonome and the transcriptome

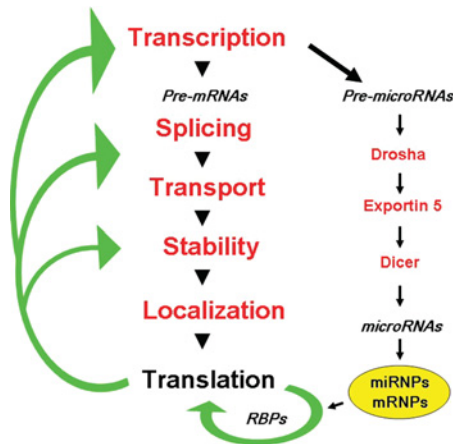
The concept of the ribonome was derived as an operational definition. Some may question whether such an environment exists and how it would differ from the transcriptome. The transcriptome is the total mRNA complement of the genome that is present in steady state at a given point of time. The ribonome on the other hand embodies the dynamic organization of mRNAs within the ribonucleoprotein infrastructure (Figure 1). Each mRNA species has multiple lives within the ribonome, and each mRNP exists in a state of activity in which the stability or translatability of mRNA components are changing in a concerted manner within each RNP in response to cellular signals. Indeed, the combinatorial PTRO model provides for each individual mRNA member of a given mRNA species to have a unique life, each of which can be regulated multiple ways as a member of more than one group of co-regulated mRNAs that form an RNA operon or regulon (Keene, 2001; Keene and Tenenbaum, 2002; Hieronymus and Silver, 2004; Moore, 2005; Keene, 2007a). However, transcriptomics does not generally reflect these diverse mRNA activities as determined by the state of the RNP but instead considers the total amount of each accumulated mRNA. For example, if there are 100 mRNAs encoding protein 'X', 25 of them may be in a translationally activated state in mRNP #1, another 50 mRNAs may be undergoing temporary translational repression within mRNP #2, and another 25 species may be stored away in a translationally silent com-

partment. The actual expression state of the total population of mRNAs would then be 25% of the theoretical amount determined by transcriptomic analysis alone since the functional state of each mRNA is not being determined. Many other examples of such a distribution can be imagined for each and every cellular mRNA. Thus, the ribonome is distinctly different from the transcriptome in part because the transcriptome does not necessarily provide functional information about the individual or the collective lives of each type of mRNA. Moreover, most transcriptional network studies base their measurements on changes of the steady state levels of mRNAs and do not account for the proportion of RNA-processing events that are acting on these mRNAs within the ribonome (Keene, 2001). Therefore, to gain more functional understanding of gene expression networks, it is essential to account for post-transcriptional co-ordination of RNA components within the ribonome.

There are also many differences between transcription and translation that account for robust regulation at the post-transcriptional level. For example, while the transcriptional machinery is confined to producing immature transcripts in the nucleus, the progression of those transcripts to translation requires many inter-connected steps of RNA processing that occur in various compartments of the cell (Figure 2). Interestingly, many RBPs participate in multiple steps along this maturation pathway. For example, ELAV/HuR, polypyrimidine-tract-binding protein, the U2AF RBPs and many hnRNP (heterogeneous nuclear RNP) proteins have a strong nuclear presence, but are believed to shuttle out of and back into the nucleus with their RNA cargo during normal cellular growth and differentiation. Many of these nucleocytoplasmic shuttling proteins are involved in nuclear RNA processing (splicing, polyadenylation and capping) and nuclear export as well as in subsequent regulation of the stability and translation of the mature mRNA targets in the cytoplasm (Piñol-Roma and Dreyfuss, 1992; Keene, 1999, 2001; Le Hir et al., 2000; Moore, 2005; Moore et al., 2006; Keene, 2007a, 2007b). Moreover, the recent demonstration that the microRNA miR-124 itself regulates the expression of the polypyrimidine tract binding protein RBP alternative splicing regulator is consistent with the dominance of the ribonome as a regulator of regulators (Makeyev and Maniatis, 2008). Indeed, a tenet of the PTRO model is that decisions

Figure 2 | Diagram of the RNA processing steps between transcription and translation showing the feedback from translation to each step in the pathway

The flow of both informational mRNAs and regulatory microRNAs from transcription and through splicing, export, stability, localization and translation is depicted. The dominance of translation in feeding back and co-ordinating events upstream of the informational flow of mRNA is depicted by green arrows. The combinatorial regulation of translation by RBPs and microRNAs is directed from within RNPs.



regarding the composition of the RNP complex and the co-ordinated fate of mRNA members of a PTRO can be determined very early in the gene expression pathway. In addition, it must be remembered that one species of mRNA is not limited to one particular pathway but instead can mature along different parallel pathways allowing multiple mRNAs to be regulated combinatorially at the levels of RNA stability and/or translation depending on the current needs of the cell (Keene, 2007a).

Similarities between the ribonome and the transcriptome

Similar to the transcriptome, that is in part determined by the modular state of the chromatin and the action of DNA-binding proteins, the ribonome is defined by the state of the RNPs that exist within the cell at any given time. Just as the RNA synthesis apparatus has modular transcriptional regulatory domains, the protein synthesis apparatus that regulates translation also has modularity. This may be counter-intuitive to many biologists because we are taught

to think of translation as a passive process that responds to any mRNA on a first come-first served basis in a more or less random Brownian environment. However, translation is a dynamic and selective process that involves modular organization and sorting of multiple transcripts through various mechanisms, including localization to the endoplasmic reticulum and potentially involves special classes of ribosomes for translating specific classes of mRNAs (Mauro and Edelman, 2002; Keene and Tenenbaum, 2002; Lerner et al., 2003; Rajasekhar et al., 2003; Nicchitta et al., 2005; Stephens et al., 2005; Komili et al., 2007; Pyhtila et al., 2008). Furthermore, translation is a highly ordered process that operates in global regulatory states and modular domains not unlike those of transcription. For example, heterochromatin is transcriptionally inactive, and likewise, there are RNPs such as processing bodies and stress granules in which mRNAs are translationally silent or turning over via specific degradative mechanisms. Interestingly, in some cases this can be a dynamically reversible process, as mRNAs may exit these particles to become translationally activated (Bregues et al., 2005; Bhattacharyya et al., 2006). In addition, post-translational modifications of both DNA-binding proteins in chromatin (Orphanides and Reinberg, 2002; Harbison et al., 2004; Aten and Kanaar, 2006; Li et al., 2008), and RNA-binding proteins in RNPs may determine the physical states of modules that co-ordinate the production of RNA and protein, respectively (Intine et al., 2003; Benjamin et al., 2006; Abdelmohsen et al., 2007; Garbarino-Pico et al., 2007; reviewed in Keene, 2007a, 2007b; Cammas et al., 2008).

Since the PTRO theory was proposed, many studies have investigated the global targets of individual RBPs and their results have supported many aspects of the PTRO model, including the main tenet that RBPs function to co-ordinately regulate functionally related messages (Keene, 2007a; Galgano et al., 2008; Hogan et al., 2008; Mazan-Mamczarz et al., 2008; Morris et al., 2008). However, dynamic regulation of the ribonome needs to be examined in order to better understand gene expression networks. As more studies of this type become available it will be important to incorporate dynamic features of the ribonome and the specific functions of PTROs into global gene expression models. Thus, to gain a complete picture of cellular gene expression, it is

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going to be crucial to fully understand the ribonome's role in shaping the overall post-transcriptional RNA network.

Intracellular co-ordination of ribonome dynamics

While most studies to date have relied on the measurement of steady-state mRNA levels to determine gene expression, it is becoming clear that these static snapshots fail to account for many regulatory events of gene expression occurring in cells. In fact, many studies looking at the relationship between the transcriptome and the proteome have reported weak to moderate correlation at best ($r = 0.2-0.6$), suggesting post-transcriptional regulation is playing an important role in co-ordinating outcomes (Gygi et al., 1999; Griffin et al., 2002; Washburn et al., 2003; Tian et al., 2004; Cox et al., 2005; Mijalski et al., 2005; Lu et al., 2006). Many studies have reported RBP target identification through ribonomic methods such as RIP-Chip (RNP immunoprecipitation-microarray) and the related CLIP (cross-linking and immunoprecipitation) procedure (Gao et al., 1994; Ule et al., 2003; reviewed in Keene et al., 2006; Keene, 2007a; Galgano et al., 2008; Halbeisen et al., 2008; Hendrickson et al., 2008; Hogan et al., 2008; Landthaler et al., 2008; Mazan-Mamczarz et al., 2008; Saint-Georges et al., 2008). All of these studies were geared toward defining the RNA contents of cellular RNPs that make the ribonome distinct from the transcriptome.

As more targets of RBPs are identified, the focus is shifting to understanding functional interactions and how RNP interactions are mechanistically manifest, as well as how they change dynamically. Rapid post-transcriptional responses to perturbations require rapid modification of RNP states and reorganization of PTROs during responses to biological stimuli (reviewed in Hieronymus and Silver, 2004). Tenenbaum et al. (2000) induced cellular differentiation of P19 preneuronal cells and noted that the mRNA components associated with the HuB protein changed. This experiment provided the first evidence that interactions between a post-transcriptional co-ordination factor and a distinct subset of associated mRNAs within the ribonome could be remodelled or crafted by internal responses of the gene expression machinery to external stimulation. It is presumed

that the induction of neuronal differentiation in these cells following the addition of retinoic acid caused specific alternations in the pathway from transcription to translation that was captured as a snapshot of the mRNA targets associated with the HuB RNP. In other words, the program of differentiation was changing at the post-transcriptional RNP level. Analysis of these associated mRNAs indicated that their presence in the RNP complex was not the result of increased transcription but instead were probably due to some mRNAs entering and other mRNAs exiting the HuB RNP. The field is now beginning to investigate the mechanisms that drive changes in mRNP composition and their underlying dynamics.

Post-translational modification of RBPs

One process that drives ribonome dynamics is cellular signalling via post-translational modification of RBPs by phosphorylation, acetylation, etc. One recently published example is the regulation of binding of HuR to the SIRT1 [sirtuin (silent mating type information regulation 2 homologue) 1] mRNA and its phosphorylation by the Chk2 kinase (Abdelmohsen et al., 2007). Chk2 is an oxidant-responsive cell cycle checkpoint kinase that is activated during cellular stress. SIRT1 is a deacetylase involved in longevity that promotes cell survival in the face of oxidative and genotoxic stress, and presumably epigenetic changes of the chromatin. SIRT1 has been shown to be a HuR target message and their binding leads to stabilization of the message and enhanced expression of the SIRT1 protein. Previous studies have shown that during cellular stress HuR is activated and stabilizes messages necessary for appropriate response to that stress (Gallouzi et al., 2000; Wang, W. et al., 2002; Gorospe, 2003; Wang et al., 2004; Bhattacharyya et al., 2006; Abdelmohsen et al., 2008). However, in this study, SIRT1 was shown to be down-regulated during cellular stress due to its dissociation from HuR after HuR was phosphorylated by Chk2. Investigation into the global effects of HuR phosphorylation showed that phosphorylation of HuR led to increased binding to certain mRNA targets (prothymosin- α , p21), and reduced binding to other targets (cyclinD, cytochrome C, cyclinA) including the SIRT1 mRNA. Mutation of the phosphorylated residues on HuR led to changes in binding affinity to these targets. In addition to Chk2, HuR has also been shown to be

downstream of a number of other kinases, including p38 and protein kinase C α as well as others (Doller et al., 2007, 2008; Kim and Gorospe, 2008). However, the precise sites and functional consequences of phosphorylation have not been identified. In addition, HuR is methylated by the CARM1 methylase in response to LPS (lipopolysaccharide) stimulation (Li et al., 2002). Thus, although global mRNA analysis of these signaling events is still being investigated, it is clear that post-translational modification of HuR can regulate its binding affinities for a variety of important growth-regulatory protein transcripts and probably affects their intracellular localization (Gallouzi et al., 2000).

Other studies have looked at the effects of phosphorylation of related RBPs. For example, when the BRF1 (butyrate response factor 1) RBP is in the unphosphorylated form, it participates in the degradation of associated messages that contain AREs (AU-rich elements). Phosphorylation of BRF1 leads to its association with the 14-3-3 scaffolding proteins, and both the BRF1 protein and its mRNA cargos are stabilized (Schmidlin et al., 2004; Benjamin et al., 2006). Phosphorylation of KRSP, another ARE regulatory RBP, can have differing effects depending on the kinase involved. Phosphorylation of KRSP by p38 kinase leads to loss of mRNA binding and stabilization of myogenic transcripts including myogenin, p21 and myoD, while Akt activation leads to KRSP phosphorylation and stabilization of a subset of messages including those encoding hnRNP F, A/B and A1, β -catenin, and the protein phosphatase PP2A (Briata et al., 2005; Benjamin et al., 2006; Gherzi et al., 2006; Ruggiero et al., 2007). These, and other examples, highlight the fact that not only is the ribonome dynamic, but that different subsets of messages can be differentially regulated within the ribonome depending on the cellular environment and signalling cascades involved (Cammis et al., 2008; Doller et al., 2008; Kim and Gorospe, 2008).

Regulators of regulators

One of the more interesting results to come out of recent ribonomic studies is that predominant among the mRNA targets within a given RNP are those that encode other regulators of gene expression, a phenomena generally described as “regulators of regulators” (Tenenbaum et al., 2000; Lopez de Silanes et al., 2004; Mesarovic et al., 2004; Penalva et al.,

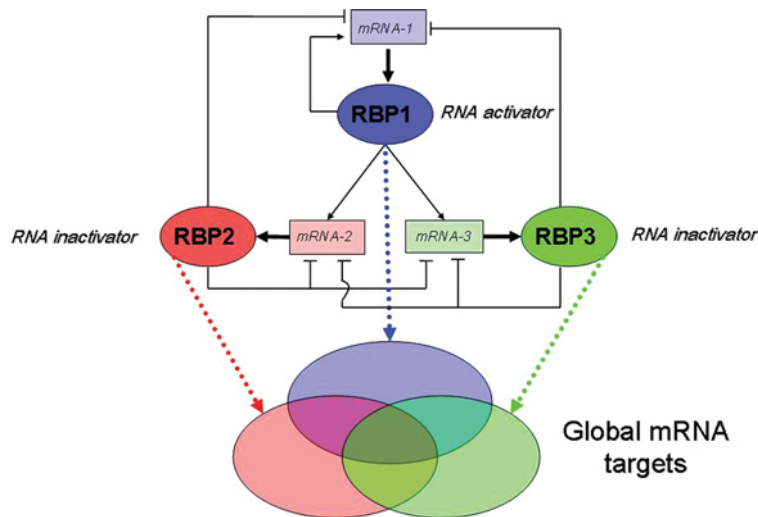
2004; Gerber et al., 2006; Keene, 2007a; Pullman et al., 2007; Morris et al., 2008; Galgano et al., 2008). These recognizable regulators of gene expression include factors that affect multiple levels including transcription, splicing, transport, stability, localization, and translation. Moreover, the participation of small non-coding RNAs and RBPs in the combinatorial trans-regulation of mRNAs encoding RBPs offers higher order modular co-ordination (Keene et al., 2006; Galgano et al., 2008; Hendrickson et al., 2008; Hogan et al., 2008; Landthaler et al., 2008). Regulation of these factors includes a series of post-transcriptional feedback loops along the pathway of gene expression (Figure 2). This interconnected network of regulators acting on one another's mRNAs ensures balanced control of a much broader set of other mRNAs that encode functionally related proteins such as those that form macromolecular complexes and signalling pathways as per the RNA operon theory (Keene and Tenenbaum, 2002).

The concept of the ribonome as a central managerial environment in biology can be visualized as a broad network of regulatory modules. As part of a subnetwork, each RBP interacts with the mRNAs encoding other regulators, thus providing a system in which each regulator can be translated only when the community of bound RBPs allows it (Figure 3). As a result of this mininetwork, these RBPs indirectly regulate the downstream targets of those regulators that it is managing. Thus, in the larger post-transcriptional network, regulators can affect global mRNA composition through their interactions with other RBPs, or by directly affecting the fate of their target mRNAs (Figure 3). Thus each of the RBPs in the system can regulate one another's mRNAs while also regulating multiple mRNA targets either individually or in concert with other RBPs within the ribonome. When a signal such as phosphorylation is directed at a specific RBP it can affect regulation of its neighbouring RBPs and/or microRNAs within the ribonome (Intine et al., 2003; Benjamin et al., 2006; Abdelmohsen et al., 2007; Garbarino-Pico et al., 2007). Therefore, the direct as well as the indirect properties of the ribonome provide a dynamic quality of multi-level co-ordination that has not been appreciated by biologists in the past.

Highlighting the regulatory potential of an RBP subnetwork, Myriam Gorospe's group recently addressed whether multiple RBPs can regulate one

Figure 3 | RBPs functioning as regulators of regulators

Model of RBPs forming a sub-network in the cytoplasm where they directly regulate the stability or translation of one another's mRNAs as an interaction balance co-ordination system (Mesarovic et al., 2004). This system is not closed but open to co-ordinating subsets of global mRNA targets (Venn ovals) dynamically in response to incoming signals as determined by the balanced regulation of each RBP in the sub-network.



another's mRNAs by investigating a group of ARE RBPs they termed "turnover and translation regulatory RBPs" (TTR-RBPs) including AUF1, HuR, KRSP, NF90, TIA-1 (T-cell-restricted intracellular antigen-1) and TIAR (TIA-1-related protein) (Pullman et al., 2007; Abdelmohsen et al., 2008). From this study it was clear that each of these six TTR-RBPs not only bound its own mRNA, but also bound to the messages of many of the other RBPs within this small group. For example, TIA-1 is regulated by mRNA stabilization by HuR, and by repression of its translation by TIAR. This study exemplifies the interconnectivity within the ribonome, as changes in two RBPs, HuR and TIAR, not only affect their own global subsets of mRNA targets, but also regulate TIA-1 protein production as part of a 'closed' TTR ribonomic sub-network, thereby affecting TIA-1's globally targeted mRNA population in the larger 'open' system. Thus, the control exerted by each member of this sub-network is proposed to provide a global co-ordination that potentially harmonizes multiple mRNAs during homeostasis as well as in response to environmental inputs.

In addition to affecting the biosynthesis of one another's proteins as part of a closed system, translation factors can also mediate regulatory events by affecting

other gene expression regulators. For example, post-transcriptional regulation of factors such as the histone deacetylase SIRT1 and other DNA-modifying enzymes could result in epigenetic changes to the genome, resulting in inheritable changes in gene expression. Furthermore, the post-transcriptional regulators of transcription and splicing also have the potential to affect the generation of and contents of a PTRO by activating or inhibiting each specific transcription or splicing factor mRNA (Figure 2). In this way, it would be possible to control the production of new members of PTROs, including microRNAs and alternatively spliced variants in the larger open system of mRNA targets. Indeed, it is well established that splicing events allow RBPs to tag the mRNAs such that subsequent post-transcriptional events, including translatability, are influenced (Le Hir et al., 2000; Moore et al., 2006). This suggests that alternative splicing of specific mRNAs can affect decisions as to which PTRO they would join; one splice variant may mark translational activation, while another might be translationally silenced. Following the appropriate signalling event, the fates of the mRNA components could change, thus feeding back to the transcription and splicing apparatus to further alter the gene expression program in a co-ordinated

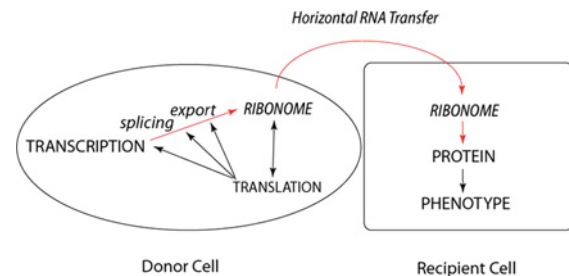
fashion. While this may be a ribonome-centric view of gene expression, it must be noted that transcriptional regulation of regulators requires that each event pass through the ribonome to either process a regulatory non-coding RNA or to translate the protein regulator (Figure 2).

Intercellular transfer of genetic information between ribonomes

Previous studies have demonstrated that conditioned media and secreted microvesicles derived from several cell types, including T-cells, alter the phenotypes of recipient cells (Hakelien et al., 2002; Fevrier and Raposo, 2004). Moreover, embryonic stem cells were shown to produce RNA-containing microvesicles that could reprogram haematopoietic progenitor cells (Ratajczak et al., 2006). Phenotypic transfer from tumour cells to monocytes by mRNA contained in microvesicles and exosomes was also reported (Baj-Krzyworzeka et al., 2006). In addition, Aliotta et al. (2007) demonstrated that RNAs contained in exosomes released from radiation-treated lung cells could transfer a lung phenotype to bone marrow cells. These findings are relevant to the classical observation of “bystander effects” on surrounding cells following irradiation. Even more interesting is the report by Valadi et al. (2007) demonstrating that both mRNAs and microRNAs are present in exosomal particles purified from mast cells. We have suggested that specific mRNAs and microRNAs organized into modular PTROs may be targeted to exosomes in order to participate in cell-cell exchange during growth, differentiation and environmental assault, including tissue repair and cell death (Keene, 2007a, 2007b). More recently, in studies of mRNAs associated with the HuR RNP in Jurkat T-cells, we found an extensive degree of overlap with mRNAs identified in mast cell exosomes by Valadi et al. (2007) and we detected HuR in exosomes purified from these cells as well (M.A. Thompson and J.D. Keene, unpublished results). These results are consistent with the possibility that exosomes could contain a HuR regulated PTRO. While much more investigation is needed, these findings could have important implications for coherent

Figure 4 | Horizontal transfer of post-transcriptional operons by exosomal particles

The intercellular transfer of ribonucleoproteins containing specific RNA subsets from a donor cell to a recipient cell is via exosomal particle release from the donor cell. This hypothesis is based upon the transfer of a coherent subset of mRNAs, microRNAs and possibly other components of the ribonome, but it has not been tested. This mechanism would provide the RNA templates and post-transcriptional regulators necessary for translational co-ordination of the transferred mRNAs in the recipient cell.



RNA transfer in many biological systems, including development and pathogenesis in mammals.

The potential of exosomes and other microparticles extruded by one cell to passage RNPs to another cell also has implications for horizontal transfer of genetic information in evolution. The PTRO model proposed that each RNA operon, representing an organized and coherent collection of information, is potentially a meta-representation of a small modular subgenome containing a few hundred mRNAs. To describe the collective functions of PTROs that contain sets of functionally coherent information in the form of RNA, the term “quasi-genome” was suggested in part for comparison with the components of some RNA viruses that contain multiple RNA genes (Keene and Tenenbaum, 2002). While still speculative, if entire PTROs are transferred between cells by modular particles, these quasi-genomes might shape the ribonome of the recipient cell and thereby alter its phenotype by bringing in RNAs and proteins that act on several levels of gene expression (Figures 2 and 4). Further studies of post-transcriptional regulation by RBPs and microRNAs within the ribonome of

Exosome: Membrane-bound vesicles (including microparticles) containing proteins, mRNAs and small non-coding RNAs that are released by cells and can be taken up selectively by surrounding cells. There is evidence that this horizontal transfer of RNA and protein can affect the phenotype of the recipient cells.

a single cell type will be needed in order to better understand the effects of their intercellular exchange on recipient cells.

As suggested previously, the idea of cell-to-cell exchange of PTROs has profound implications for the origins and evolution of viruses as well as cells (Keene, 2007a). Gould et al. (2003) suggested that exosomes augment HIV infection by assisting virus entry and subsequent infectivity. We proposed that PTRO-containing exosomes could be ancestors of RNA viruses by providing groups of mRNAs that encode RBPs that could ultimately serve as viral genes and viral proteins respectively. That RNA operons may be evolutionary precursors of RNA viruses is based in part on the finding in many labs that RNA operons identified to date contain mRNAs encoding many other RBPs (Keene, 2007a, 2007b). Thus, not unlike RNA viruses that contain predominately RNAs encoding RNA-binding and processing factors, RNA operons have the potential to become 'selfish' and parasitic in order to gain fitness for an independent existence by evolving toward self-replication (Keene, 2007a, 2007b). This may have helped give rise to retroviruses as 'non-professional' mediators of horizontal gene transfer, as opposed to the naturally shed cell entities that we call exosomes from which they may have been derived.

While the idea of exosomes transferring PTROs from cell to cell is intriguing, many questions remain, including the specificity of cell-to-cell transfer, as well as how exosome production and content is regulated. Recently, Arnold Levine and co-workers suggested that a novel function of the p53 tumour suppressor protein is to stimulate exosomal secretion (Yu et al., 2006). Given that p53 responds to stress signals by affecting transcription, genes can be activated that affect secretion, many of which are contained in exosomes. Following H460 cell DNA damage, an analysis of secreted proteins indicated that p53-activated gene products were found in the culture medium. The investigators demonstrated that these p53-activated proteins exited the cells as components of exosomal vesicles. A specific gene product, TSAP6, was found to enhance exosome production from p53-activated cells and these exosomes transferred proteins to other cells. These experiments did not investigate

whether RNAs were contained within these exosomes, but as noted above, recent studies from several laboratories suggested that RNAs are cargoes of exosomes as well (Baj-Krzyworzeka et al., 2006; Ratajczak et al., 2006; Aliotta et al., 2007; Valadi et al., 2007). Interestingly, the HuR RBP that forms one of the best documented mammalian PTROs activates the translation of p53 protein in UV-treated cells, making it a good candidate for an intermediate regulator of exosome production (Mazan-Mamczarz et al., 2003; Keene, 2007a).

The classical model for the origins of retroviruses suggested that moveable genetic elements were their ancestors, and many endogenous human retroviruses have since been identified as presumed remnants of exogenous retroviruses (Temin, 1980; Wang et al., 2007). The well-documented exchanges between retroviral sequences and host genomic sequences are believed to be a major driving force of evolution. The recent fascinating discovery that the human p53 transcriptional network may have been shaped during the split between Old-World and New-World monkeys approx. 40 to 60 million years ago by a temporal wave of exchange of endogenous retroviruses raises the question of how these primate exchanges were motivated (Wang et al., 2007). Could it be that particle exchanges of RNAs or DNAs between cells were mediated by exosomal particles and that these events represent evolutionary tracks of retrovirus emergence that accelerated evolution and helped craft the human genome? Elucidating the functional organization of mRNAs and microRNAs within microparticles and exosomes, and the effects of their horizontal transfer to recipient cells has the potential to reveal new mechanisms of cell–cell communication at the level of RNA.

Conclusions

For several years we have maintained that transcriptomic data represents a very incomplete picture of global control and co-ordination of gene expression (Keene, 2001, Keene and Tenenbaum, 2002, Keene, 2007a). Indeed, gene expression networks as derived using transcriptomic data alone do not distinguish transcriptional events from post-transcriptional events, yet both are represented in such data sets,

Control and co-ordination: Control is a strong, directed effect of a transacting factor on a single RNA component, whereas coordination is a multiplicity of generally weaker effects that orchestrate or harmonize RNAs. Thus these are different but overlapping aspects of RNA regulation.

and this can lead to misinterpretation of regulatory networks. The intrinsic modularity of the PTRO model includes both control and co-ordination network qualities that may help determine underlying mechanisms of translation where operational processes cycle back to earlier steps in the pathway of gene expression (Figure 2).

In classical molecular biology, 'control' involves dictation of one-on-one events, whereas the term 'co-ordination' describes a distributive process that harmonizes global events by motivating multiple control functions (Mesarovic et al., 2004). However, the term 'regulation' has been used to encompass both single and multiple controlling and/or co-ordinating events of unknown configuration that involve any number of combined molecular processes, presumably leading to global gene expression outcomes. Therefore, it is important to understand these separate but inter-related aspects of gene regulation and to account for both kinds of information when interpreting and constructing network models.

It is clear that biogenesis of the proteome determines cell fate, and strongly affects the performance of the surrounding 'bystander' cells in multicellular organisms. Moreover, every transcription regulator must be translated and every translation factor must be transcribed. Therefore, at the very least eukaryotic cells have two intercommunicating and interdependent gene expression networks that are likely to be synergistic and they must both be understood before a comprehensive unified model can be formulated. We view the ribonome as a layered system of modules and circuitry that provides control, co-ordination and bounded autonomy across gene expression networks, much of which depends on the cellular localization of RNP particles and granules (Mesarovic et al., 2004; Davidson and Ellington, 2007). Each decision as to when and where to translate discrete subsets of functionally related mRNAs is exceedingly important for co-ordinating coherent outcomes, and is essential for the performance of cellular homeostasis, responses to environmental signals and differentiation.

Finally, we view the ribonome as the dominant environment of gene regulation. The evidence and reasoning as outlined in this article includes: (i) the high abundance of RNA-binding and -processing factors in eukaryotes (Keene, 2001); (ii) the ability of RBPs to locally determine one another's biosynthetic role as regulators of regulators (Penalva et al., 2004;

Keene, 2007a, 2007b; Pullman et al., 2007; Morris et al., 2008; Galgano et al., 2008; Saint-Georges et al., 2008); (iii) the fact that there are multiple copies of each monocistronic mRNA, each of which can be regulated independently or in combination with others (Keene and Tenenbaum, 2002; Moore, 2005; Halbeisen et al., 2008; Hogan et al., 2008); (iv) the well-established responsiveness of RNA stability and translation to external signals mediated by post-translational modifications (not unlike transcription) (Intine et al., 2003; Abdelmohsen et al., 2007, 2008); and (v) the fact that many regulators of transcription, splicing and RNA export are primary mRNA targets of translation itself (reviewed in Keene, 2007a). This latter point is highlighted by the fact that transcriptional regulators must be translated in the cytoplasm, making a transcriptional 'regulators of regulators' model dependent upon the downstream performance of the entire post-transcriptional program that then produces new transcription factors or chromatin-modifying factors that must be transported back to the nucleus. On the contrary, translational RBP regulators can directly determine which regulatory proteins are able to be made for feedback to transcription, splicing, export, stability and translation itself, because their mRNAs are present as mRNPs in the cytoplasm; in many cases these RBPs are bound to one another's mRNAs, and thus, have an opportunity to directly 'vote' on one another's fate.

While one may argue that this 'chicken or egg' scenario of transcription versus translation is not surprising, prevailing dogma places transcriptional control at the centre of gene regulation (control and co-ordination), and relegates translation to a more passive role in which control points operate for individual mRNAs but co-ordination is not considered (Keene, 2001). We have challenged this traditional model of gene expression because data supporting the PTRO model is based on extensive co-ordination of gene expression by RBPs and non-coding regulatory RNAs, suggesting at least an equal, and probably more agile, role for post-transcriptional co-ordination. This less traditional model regarding the importance of post-transcriptional events is consistent with the RNA world view of evolution in that the protein biogenesis machinery is thought to have preceded the acquisition of a nucleus and the appearance of DNA

(Margulis, 1970; Martin and Koonin, 2006). Whether post-transcriptional processes overall have retained a dominant operational mode in evolution is yet to be substantiated but at the very least, future models of global gene expression need to account for the extensive amount of post-transcriptional co-ordination that has now been widely demonstrated (Keene, 2001; Keene and Tenenbaum, 2002; Hieronymus and Silver, 2004; Moore, 2005; Keene, 2007a, 2007b; Halbeisen et al., 2008).

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