'Cradle–to–grave' regulation of mRNA fate

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Microarray studies in *Saccharomyces cerevisiae* have set the benchmark for genome-wide analyses, available data-sets covering practically every stage of gene expression from DNA-binding by transcription factors to mRNA export, sub-cellular localisation, translation and decay. A theme to emerge from such data has been the prevalence of coordinate gene regulation. Thus, gene modules or 'regulons' are well recognised at the level of gene transcription and the activity of transcription factors provides an obvious molecular explanation for such coordination. More surprising was the organisation of mRNAs into co-regulated 'post-transcriptional operons' [1]. RNA-binding proteins (RBPs), but also ribonucleoprotein (RNP) complexes involving noncoding RNA [2], have been proposed as the conceptual equivalent of transcription factors at this level.

An example is the environmental stress response (ESR) regulon; ~900 genes identified by transcriptome profiling that are co-regulated in many types of stress [3]. While part of this response is explained by changes in gene transcription, microarray-based measurements of mRNA decay revealed a major contribution to this signature by coordinated changes in transcript stability [4]. Thus, eukaryotic gene expression patterns arise as a consequence of the action of regulatory networks driven by DNA binding proteins and those driven by RBPs (Figure 1). The *S. cerevisiae* genome encodes hundreds of distinct RBPs, which are expected typically to bind several or many RNA species. Individual RBPs have been shown to determine the fate of mRNAs at all post-transcriptional levels. Cells may deploy the intricate combinatorial action of RBPs to reinforce the modular pattern set up by transcriptional output, or alternatively to create further diversity by directing mRNAs into differential cytoplasmic fates.

Several 'ribonomic' approaches were developed in yeast, ultimately to help establish a systems biology of post-transcriptional control [5]. They all combine a biochemical step to fractionate mRNAs based on a parameter that is indicative of their regulatory fate, with subsequent microarray analysis.

RNP-immunoprecipitation array (RIP-Chip) analysis

Understanding RBP networks requires knowledge of the mRNA targets of the RBPs. How these can be found is illustrated by a recent study that used affinity purification of RNPs followed by microarray detection to identify mRNAs bound by each of five pumilio-homology domain family (PUF) RBPs in *S. cerevisiae* [6]. Striking results were a marked selectivity towards binding of 40–220 mRNAs for each PUF protein and clear enrichment of certain gene ontology terms within each target group. For instance, nuclear transcripts encoding mitochondrial proteins interacted selectively with Puf3 and Puf4 preferentially bound mRNA encoding nucleolar proteins. Implicit in the interpretation of this study is that the PUF proteins will influence the cytoplasmic fate of transcripts in terms of localisation, translation and stability, as shown for individual examples [7].

Translation state array (TSA) analysis

A major target of post-transcriptional gene regulation is mRNA translation into protein [8]. The translation state of an mRNA can be inferred from its partitioning between actively translating ribosomes (polysomes) and non-translating ribonuclear particles (RNP) during density gradient centrifugation of cellular extracts. We and others have developed genome-wide versions of this classic approach (Figure 2), and applied them to the steady-state transcriptome of exponentially growing yeast, as well as to cells responding to several external stimuli [5,9]. A global observation in untreated cells was that the number of ribosomes per mRNA increased with the length of coding sequence. Overall packing density was well below maximum, consistent with initiation as the rate-limiting step in translation. Unexpectedly, ribosome density decreased strongly with increasing coding region length [10]. We have simultaneously monitored transcriptome and translation

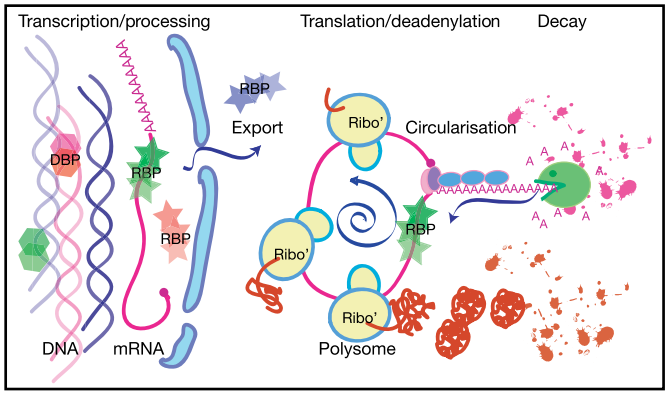

Figure 1. Regulation of mRNA fate. DNA binding proteins (DBPs) in the nucleus orchestrate transcriptome synthesis. RBPs determine the subsequent transcript fate by influencing processing, export, localisation, translation, deadenylation and decay. Shown in schematic is an mRNA exported from the nucleus with star-shaped RPBs. Cap- and poly(A) tail-binding proteins in the cytoplasm circularise the transcript to stimulate multiple rounds of translation by ribosomes (ribo'). Deadenylation eventually triggers mRNA decay.

state changes in cells responding to treatment with the TOR kinase inhibitor rapamycin, or heat shock. In both treatments there were strong tendencies for mRNAs that are induced in the transcriptome to also be more efficiently translated; similarly, mRNAs with reduced levels after treatment typically also show lower translational fitness [11]. This type of 'potentiated' gene regulation has since been noticed in several other TSA studies and the mechanistic basis for these patterns is a focus of our current research.

Polyadenylation state array (PASTA) analysis

The mechanistic switches that drive an mRNA from polysomes to the non-translating pool are 'hot' topics of research [8], not least because of the recognition of microRNA as pervasive translational regulators [2]. Control is frequently achieved through an interference with the function of the major 'promoters' of mRNA translation, the 5' cap structure and 3' poly(A) tail [12]. The poly(A) tail is of particular interest, as dynamic variation in its length affords a tuneable mode of mRNA-specific control [13]. Furthermore, work by Jörg Heierhorst in Melbourne and others, clearly linked the activity of deadenylases to cell cycle control [14-16]. This prompted us to adapt a method to separate mRNA by tail length, through thermal elution from poly(U) sepharose, for analysis of fractions by microarray (Figure 3). We performed such PASTA experiments for the transcriptomes of *S. cerevisiae* and *S. pombe* (in collaboration with Jürg Bähler, Hinxton, UK). These data reveal interesting relationships between tail length and other mRNA characteristics and suggest cellular processes that depend on poly(A) tail length control. We are pursuing these leads in ongoing research.

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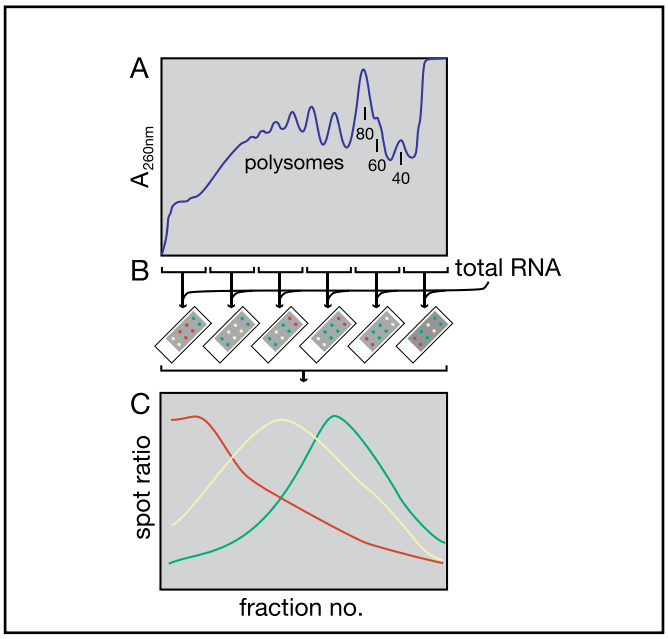

Figure 2. TSA. Sucrose gradient centrifugation of cell extracts separates actively translating polysomes from non-translating ribosome subunits (40&60) and monosomes (80). **(A)** Fractions are collected and RNA isolated from each fraction is compared by microarray to unfractionated RNA from the same culture, resulting in a genome-wide snap-shot of mRNA translation. **(B)** The resulting microarray data-set represents a compendium of gradient profiles for all detectable mRNAs. For instance, a prototypical well-translated mRNA that is enriched in polysomes is indicated by the red trace, whereas the green trace represents an mRNA enriched in the monosome fraction indicating poor translational efficiency **(C)**.

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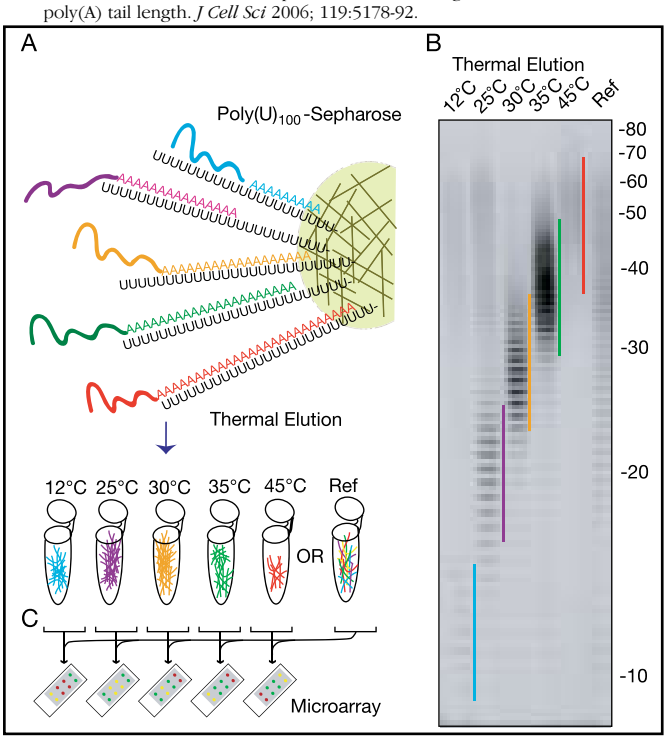

Figure 3. PASTA. Total RNA is bound to poly(U)100-Sepharose, washed and eluted batch-wise at indicated temperatures **(A)** To show that each temperature step enriches mRNAs of defined poly(A) length, a portion of the RNA is 3' end-labelled with ^{32}P and incubated with RNase A&T1 leaving only A-tails intact; these are then separated by 16% Urea-PAGE and visualised by autoradiography **(B)**. Samples in each fraction are compared to reference mRNA by microarray **(C)**.