In conclusion, we have described the concept of real temperature optima for enzymes, and the means to distinguish the phenomenon from cases of apparent optima in which protein instability is a major factor in the temperature-dependence of enzyme activity. The question now is about the nature of the structural changes between active and inactive forms of the enzyme, and how these might be determined. Furthermore, if real temperature optima exist there is an additional consideration for the enzyme engineer; for example, engineering an enzyme to operate at high temperatures might no longer be merely a question of creating a more stable variant: thermoactivity might have to be introduced in addition to thermostability.

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Translation control: bridging the gap between genomics and proteomics?

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mRNA profiling enables the expression levels of thousands of transcripts in a cell to be monitored simultaneously. Nevertheless, analyses in yeast and mammalian cells have demonstrated that mRNA levels alone are unreliable indicators of the corresponding protein abundances. This discrepancy between mRNA and protein levels argues for the relevance of additional control mechanisms besides transcription. As translational control is a major mechanism regulating gene expression, the use of translated mRNA in profiling experiments might depict the proteome more closely than does the use of total mRNA. This would combine the technical potential of genomics with the physiological relevance of proteomics.

Proteins, rather than genes or mRNAs, represent the key players in the cell. The proteome (i.e. the complete set of proteins encoded by the genome[1]) determines the cellular phenotype and its plasticity in response to external signals. Expression levels of a protein depend not only on transcription rates of the gene, but also on additional control mechanisms, such as nuclear export and mRNA localization[2], transcript stability[3], translational regulation[4] and protein degradation[5–7]. Moreover, both the activity and the function of proteins can be altered, mainly through post-translational modifications (e.g. glycosylation and phosphorylation) or proteolytic cleavage[8]. Transcriptional and post-translational regulation have attracted much attention, whereas the regulation of protein levels by translational control has often been either neglected or underestimated[9]. However, in recent years, interest in the mechanisms controlling the activity of the translation machinery – during development, in response to extracellular stimuli, following viral infections or in disease – has notably increased[10]. The best-understood mechanisms controlling translational efficiency are those that act at the level of initiation, which involve structural elements within particular mRNAs, modifications of components of the initiation machinery or the regulated association of the initiation machinery with other proteins that affect the activity of the complex[11]. Indeed, rather than influencing a few individual mRNAs, translational control is a widespread mechanism for regulating gene expression. This was first demonstrated upon oocyte fertilization[12,13] and reticulocyte maturation[14]. More recently, it has been shown that translational control affects ~20% of the genes regulated upon T-cell activation[15]. Thus, an idea is emerging that the relevance of mRNA profiling data might be improved if the level of translational regulation is also taken into account.

Do mRNA expression levels faithfully reflect protein abundance?

A recurring criticism to the use of mRNA expression profiling in characterizing cellular phenotypes has been that the transcriptome does not faithfully represent the proteome[16]. A limited number of reports have compared the steady-state levels of proteins with those of their corresponding mRNAs. Results from these studies have suggested that mRNA abundance is a poor indicator of the levels of the corresponding protein[17–20]. As it is the proteome that determines cell phenotype, this disparity between protein and transcript levels might lead to misinterpretation of mRNA profiling results.

To date, the two most extensive analyses correlating mRNA and protein expression levels have both been

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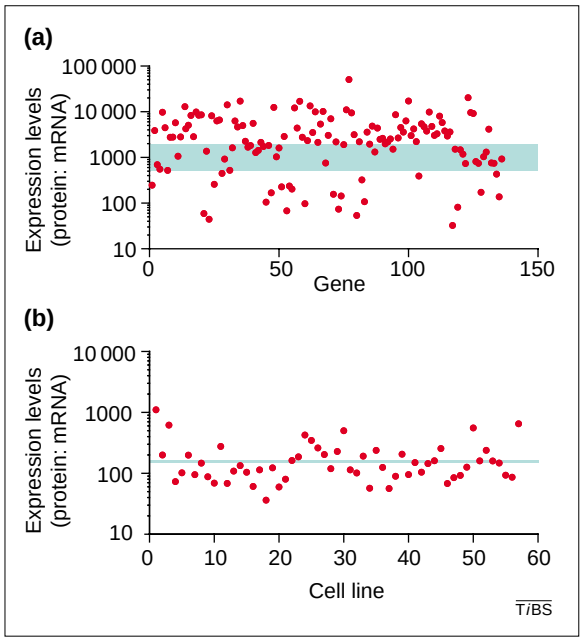

Fig 1. Weak correlation between mRNA and protein levels in eukaryotes. (a) The correlation between the expression levels of 136 *Saccharomyces cerevisiae* mRNAs and their corresponding proteins[19] is depicted as protein:mRNA ratio (arbitrary units) against a running index. Numbers of transcripts per cell for each mRNA were deduced from SAGE (serial analysis of gene expression) data[42]; protein levels were determined after separation of samples in 2D gels, followed by quantification and identification of the spots[19]. The data reveal a poor correlation between mRNA and protein abundance (r = 0.57). Indeed, 72% of the genes analysed fall outside a confidence interval (aqua), being twofold higher or lower than the average ratio. Protein abundance can vary more than 20-fold for a given expression level of a particular mRNA. Conversely, for a given amount of protein, the expression of the corresponding transcript can differ by up to 30-fold. (b) The correlation between mRNA and protein abundance for glutathione-S-transferase in 57 human cell lines derived from nine different tissues[16,17] is represented as in (a). In this case, the correlation is even lower (r = 0.43) and there is a greater than 40-fold variation with respect to the average protein:mRNA ratio (aqua line). These data, together with additional examples[18,20], clearly demonstrate that protein expression cannot be reliably predicted from mRNA expression levels alone.

conducted in yeast[19,20]. Both of these analyses revealed that the abundance of the corresponding protein, for a given mRNA expression level, might vary by up to 30-fold (Fig. 1a) and vice versa. Interestingly, the correlation was even lower for the less abundant proteins taken into account, despite the fact that in both reports only abundant proteins were analysed. In mammalian cells, direct comparisons of mRNA and protein levels have been performed either for several genes in one tissue[18], or for one gene product in many cell types[17] (Fig. 1b). In both cases there was a poor correlation (coefficient <0.5) between mRNA and protein levels, which was even weaker in tumour cell lines[17]. Taken together, the available data indicate that the deduction of protein abundance from mRNA expression levels alone appears unreliable, thus seriously questioning the relevance of using mRNA profiling data to elucidate cell phenotypes.

As mentioned above, control of gene expression not only involves transcription and mRNA stability, but also control at other levels such as mRNA processing, nuclear export, translation and protein degradation[3,4,9,21]. Therefore, the observed disparities between mRNA and protein abundance were not unexpected[16,22] and argue for the importance, when performing expression profiling studies, of taking these additional levels of control into account.

Technical limitations to proteome analysis

As a consequence of the discrepancies between the levels of mRNAs and their proteins, the most meaningful approach to describe cell phenotypes would be an exhaustive, quantitative analysis of the proteome. Proteomics, a term covering all the technology currently available to analyse global patterns of gene expression at the protein level[23], usually involves separation of proteins from cells or cell fractions in 2D gels, followed by identification of individual spots by mass-spectrometry. Recent applications of this technology include the identification of marker proteins in several carcinomas and haematological malignancies, the characterization of proteins deregulated in heart diseases, the evaluation of drug toxicity and the detection of proteins involved in Creutzfeld–Jacob disease[23]. Proteomics enables the quantification not only of protein steady-state levels (by staining), or synthesis and turnover rates (by pulse-labelling), but also of post-translational modifications such as phosphorylation and glycosylation[8,16,24]. A particular strength of proteomics is that all of these approaches can be applied together to extensively characterize a biological system, as demonstrated in a recent study on early events in apoptosis[25].

Unfortunately, proteomics still has serious drawbacks, the most severe of which is the limited resolution of 2D gels. This resolution enables the separation of, at most, 3000–4000 individual proteins and is consequently strongly biased against the identification of less abundant proteins[8,26]. Indeed, even the most recent fluorescent staining methods only cover a dynamic range of four to five orders of magnitude, whereas the proteome appears to span more than seven[16,23,27]. In genomics, it is possible to select against highly expressed mRNAs by subtractive approaches[28] and to amplify low abundance transcripts by PCR-based methods. By contrast, no equivalent techniques are currently available for proteomics. As a consequence, many important regulatory molecules, such as cytokines and their receptors, signal transduction proteins, cell-cycle regulators and transcription factors, usually fail to be detected[25]. Another difficulty is the proper detection of hydrophobic, basic, very small or very large proteins (the last of which represent 11% of the proteins from *Saccharomyces cerevisiae*[8]). In addition, there are still major problems with quantification of spots, besides the selectivity of protein stains, because of the automation of this process by image analysis. Such automated quantification impedes the correlation of protein expression data from one cell system to another, despite the use of advanced computer algorithms[8]. Thus, it appears that proteomics would be best used to characterize the protein content of subcellular

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fractions (such as plasma membrane, organelle, cytosolic and nuclear fractions), rather than to depict the whole proteome[8,23,27]. The various drawbacks imposed by 2D-gel electrophoresis call for alternative separation methods[24,27], some of which are currently being developed[29,30].

Expression profiling of polysome-bound mRNAs: a closer representation of the proteome?

The above considerations suggest that expression profiling data would be more meaningful if mRNA samples could be enriched for transcripts that are being translated. This can be achieved by fractionation of cytoplasmic extracts in sucrose gradients, enabling the separation of free mRNPs (ribonucleoprotein particles) both from mRNAs in ribosomal pre-initiation complexes and from mRNAs fully loaded with ribosomes (i.e. polysomes). As only polysomes represent actively translated transcripts, this fraction should be directly correlated with the set of *de novo* synthesized proteins in a particular cellular state[31]. This hypothesis has been verified experimentally for individual mRNAs; hybridization of northern blots with specific probes revealed the distribution profile of specific mRNAs between ribosome-free and polysome-bound fractions (Fig. 2). Comparison of these profiles then enabled the determination of the translation efficiencies, which are characteristic for each transcript in a cell[32,33]. In addition, changes in the distribution of a given mRNA indicate how this translational efficiency can vary under different conditions[34,35]. Because it is generally accepted that translational control predominantly occurs at the initiation step[4,10,36], the number of mRNA molecules engaged in polysomes should be a robust indicator of the synthesis rate of the corresponding protein.

By using polysome-bound mRNA in expression profiling experiments, several groups have directly identified targets for translational control[37–39]. The methods applied in these studies generally involved the fractionation of cytoplasmic extracts through sucrose gradients. Thereafter, pools of fractions corresponding to the polysome-bound transcripts were used to generate probes for array hybridization. The first published report demonstrated the selectivity of translational control, identifying ~1% of the analysed genes as translationally regulated transcripts in human and murine fibroblasts upon serum-induced mitogenesis[38]. Using a similar approach, it was shown that mRNAs containing internal ribosome entry sites represent ~3% of all expressed transcripts[37]. In this system, polysome-bound mRNAs from poliovirus-infected cells were used for profiling because poliovirus degrades eIF4G (eukaryotic initiation factor 4G), a key component of the ribosomal machinery that initiates translation by 5′ cap recognition, so the mRNAs that remain polysome-bound should be translated via a cap-independent mechanism. Finally, polysome-bound mRNA profiling revealed that >10% of the mRNAs analysed were translationally regulated upon the activation of primary human T lymphocytes[39].

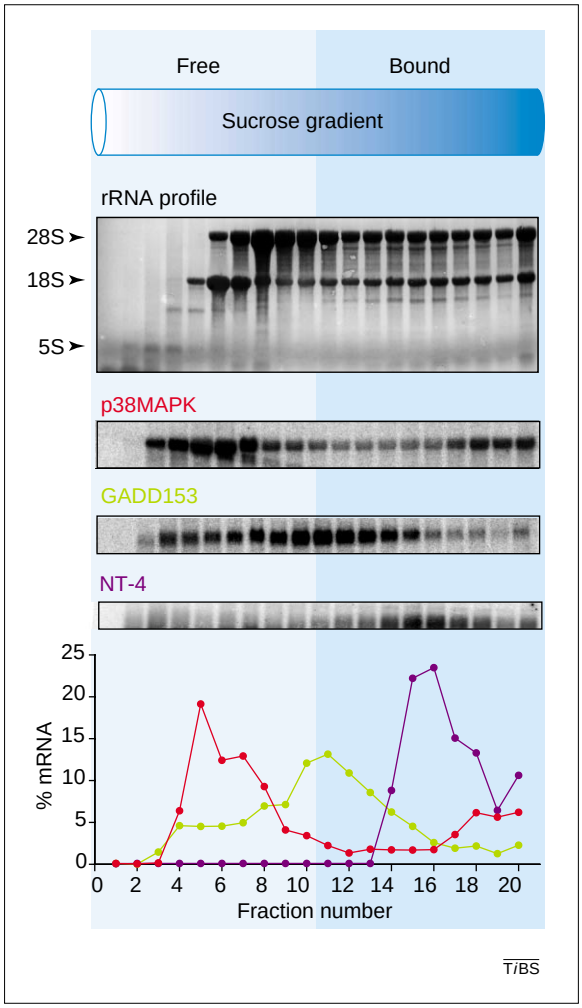

Fig. 2. Different mRNAs are translated with widely different efficiencies. Cytoplasmic extracts from primary human activated T cells were fractionated in sucrose gradients, and the RNA from each fraction analysed by northern blotting. The ribosomal RNA distribution profile (28S, 18S and 5S rRNA; indicated by arrowheads in the top panel) enables the distinction of fractions comprising mRNPs and mRNAs engaged in pre-translational complexes (fractions 1–10; light-blue area, designated as 'free'), and those containing the polysome-bound mRNAs (fractions 11–20; dark-blue area, labelled 'bound'). Hybridization with probes specific for the mRNAs encoding p38MAPK (red), GADD153 (green) and NT-4 (purple) revealed extensive variations in mRNA distribution in sucrose gradients[39]. In the corresponding distribution profiles (bottom), intensities for each fraction are plotted as a percentage of the total signal on the filter for a given mRNA to facilitate comparison. The differential distribution of these mRNAs implies different translation efficiencies. This phenomenon appears to be much more common than previously anticipated, and is not restricted to particular transcripts, cell types or species[15,37–39]. Abbreviations: GADD153, growth arrest and DNA damage; MAPK, mitogen-activated protein kinase; NT-4, neurotrophin 4.

Reliability of profiling using polysome-bound mRNA is a crucial issue for this type of analysis because the populations to be compared might have different complexities and abundances. Normalization can be conducted using, for each sample, either similar amounts or similar volumes of mRNA, and by monitoring either the ratio of polysome-bound mRNAs to total mRNAs, or the changes in the polysomal populations as compared with a reference sample[37,40].

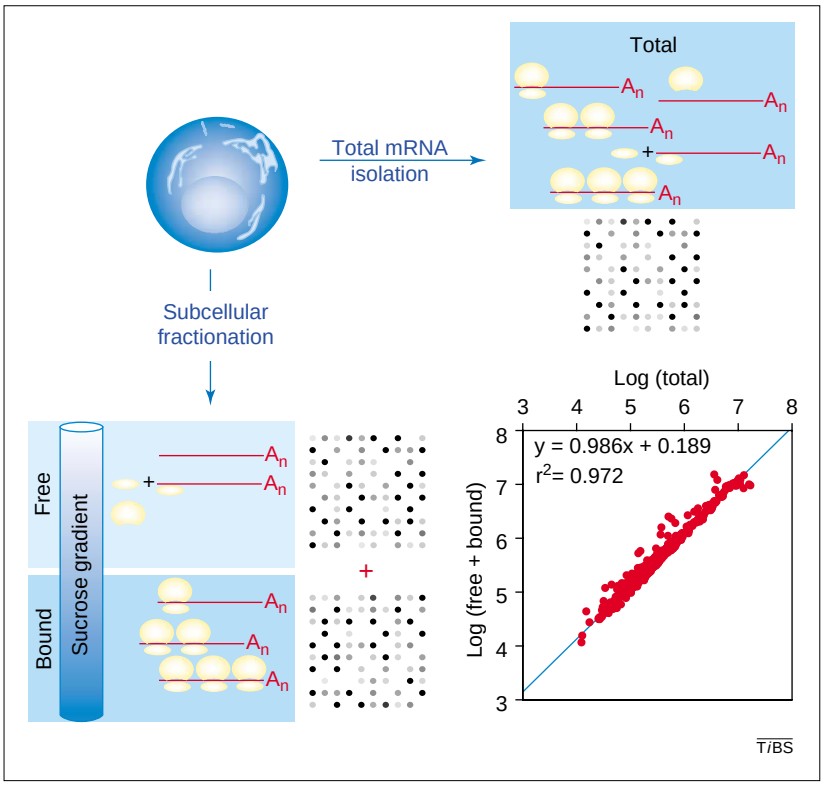

Fig. 3. Polysome-bound mRNA expression profiling can be quantitated both reproducibly and accurately. Total mRNA, and ribosome-free and polysome-bound mRNA populations, were isolated from a T-cell clone, the last two via fractionation through a sucrose gradient. These three populations were then analysed on arrays. For each cDNA, the sum of the hybridization signals obtained with polysome-bound plus ribosome-free targets was compared to the signals from the total mRNA sample. Statistical analysis indicated that the signal for polysome-bound plus ribosome-free mRNA closely matched the one of total mRNA (Ref. 41), thereby demonstrating the accuracy of this separation method.

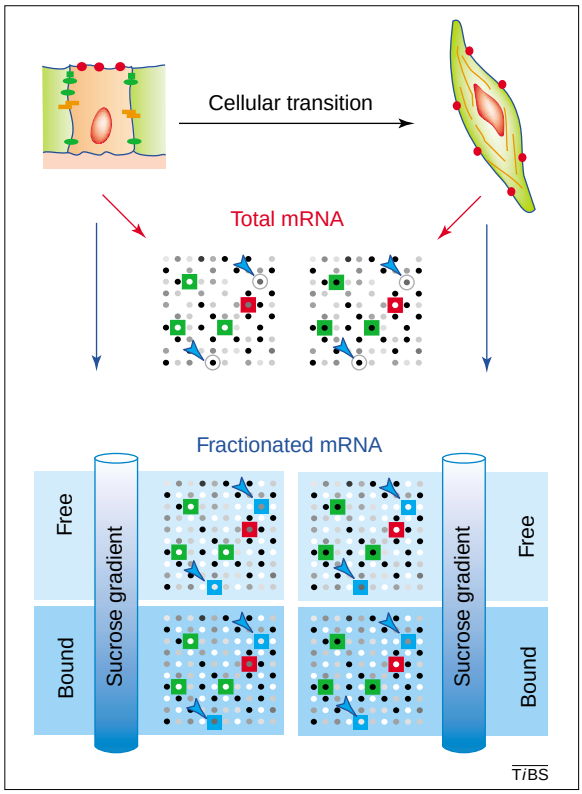

Fig. 4. Polysome-bound mRNA expression profiling detects both transcriptionally and translationally regulated genes. Arrays are frequently used to analyse changes in expression patterns during a physiological transition (e.g. epithelial cells undergoing mesenchymal transition[43]). In conventional experiments, profiling total mRNA from such cell pairs (red arrows) identifies genes being either transcriptionally induced (green) or repressed (red) during the transition. However, translationally regulated mRNAs (grey circles) are not detected. By contrast, fractionating total mRNA into polysome-bound and ribosome-free mRNA populations (blue arrows) enables the detection of translational changes (bottom half of the figure). mRNAs redistributing (blue squares) from the ribosome-free towards the polysome-bound pool (translational activation), or vice versa (translational repression), will display different hybridization signals depending on whether ribosome-free or polysome-bound targets are used. It is important to note that hybridization signals for transcriptionally regulated mRNAs (red, green) will be uncovered exactly as in standard profiling using total mRNA.

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Using filter arrays, reliability was assessed by hybridization of two RNA populations (ribosome-free and polysome-bound), with different inherent complexities and abundances, both originating from the same cytoplasmic mRNA population. For each transcript, the sum of the signals obtained from the ribosome-free plus the polysome-bound populations was similar to that obtained from the original cytoplasmic RNA (Ref. 41; Fig. 3). Hence: (1) expression profiling using polysome-bound mRNAs generates reproducible, quantitative data; (2) total mRNA cannot properly account for the translated mRNA population; and (3) the comparison between the total and the polysome-bound mRNA populations enables transcriptional and translational regulation to be distinguished.

Expression profiling of polysome-bound mRNA has also been used to analyse epithelial cells during mesenchymal transition (M. Jechlinger and N. Kraut, pers. commun.) and murine erythroid progenitors either undergoing terminal differentiation or responding to cytokines (M. von Lindern, pers. commun.) In both cell systems, although the majority of all regulated genes were regulated at the transcriptional level, a large fraction (10–20%) were translationally controlled. Moreover, the analysis of total mRNA showed that these genes were not subject to substantial transcriptional regulation, and so would have been missed in conventional mRNA expression profiling (Fig. 4). Several of these gene products were analysed by western blotting, and the observed changes in protein levels confirmed that they were translationally regulated. Moreover, for these examples, a highly significant correlation was observed between the levels of protein and the corresponding polysome-bound mRNA.

The above reasons suggest that, for profiling analysis, polysome-bound mRNA (which integrates all events contributing to the final rate of protein synthesis, including transcription, maturation, export, stability and translation) should correlate more closely with protein synthesis than does total mRNA. Thus, polysome-bound mRNA profiling should provide a closer representation of the proteome than does profiling of total mRNA. Although supported by data (M. Jechlinger and M. von Lindern, pers. commun.), and by measurements of total protein synthesis rates and overall polysome-bound mRNA

levels[31], this hypothesis remains to be proven on a proteome-wide scale. This would require identification of a large number of proteins in 2D gels, determination of their synthesis rate by radioactive labelling, and correlation with results from polysome-bound mRNA profiling. The rapid development of mass-spectroscopy to generate large quantities of peptide sequence from proteins that are available naturally only in the low femtomolar range, bears the promise to make such analyses feasible very soon.

Polysome-bound mRNA profiling cannot be used to study changes affecting protein levels by proteolysis, post-translational modifications, subcellular localization or protein degradation; such studies will only be accessible by proteomics. However, this translational profiling might narrow, at least in part, the gap between genomics and proteomics (Fig. 4). Furthermore, the availability of hundreds of genes that are exclusively or predominantly regulated by translation will greatly facilitate a comprehensive analysis of the mechanisms involved in this type of regulation.

Conclusion

Polysome-bound mRNAs obtained by sucrose-gradient fractionation can be used for quantitative analysis in mRNA profiling experiments. This methodology integrates every level of regulation from transcription to translation. It also combines the technical potential offered by genomics in terms of high throughput, feasibility, reproducibility, sensitivity, target identification, and adaptability to new cell systems, with the physiological relevance of proteomics analysis. This translational profiling technique does not detect alterations in post-translational events. Nevertheless, it promises to make an important contribution towards characterization of the proteome, and could therefore significantly help bridge the gap between genomics and proteomics.

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