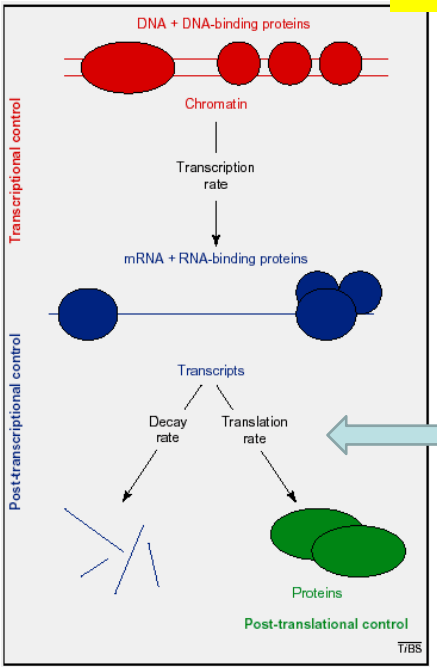
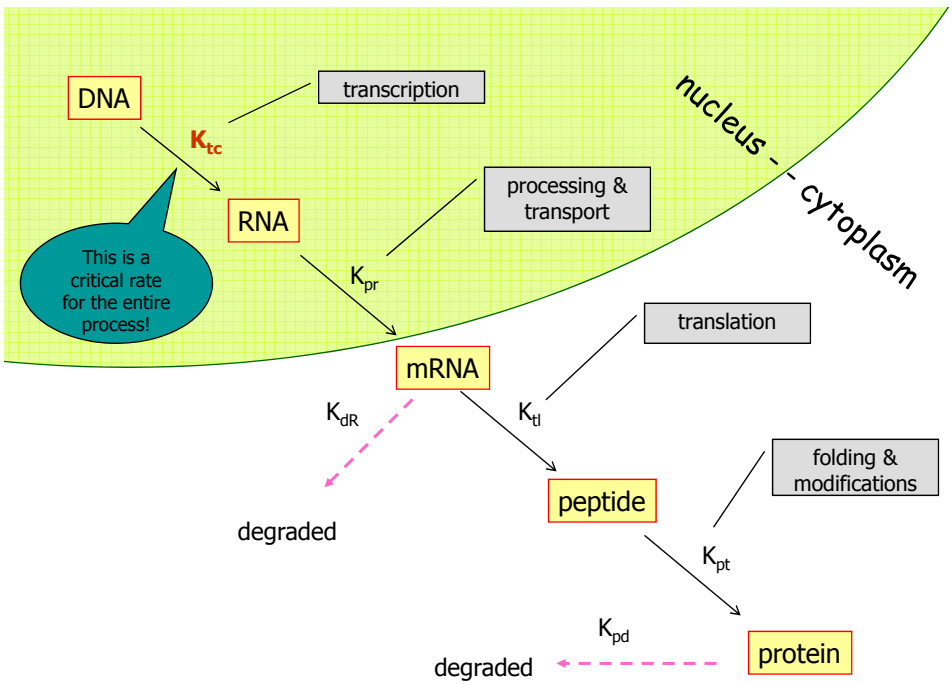
- Post-transcriptional regulation & proteomics

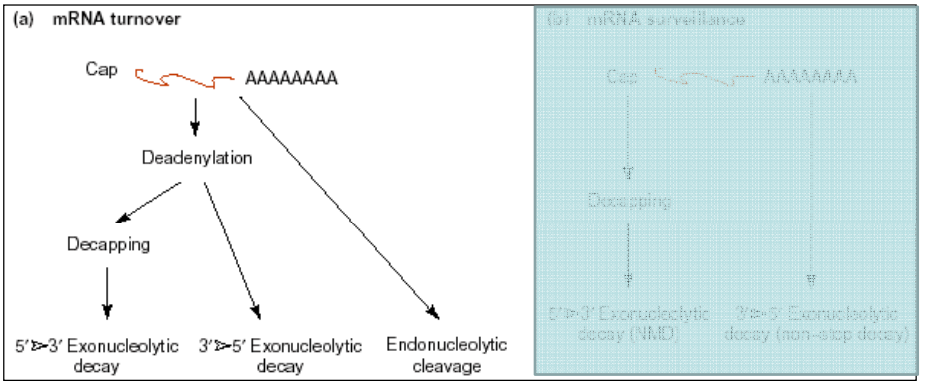


POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION

mRNA TURNOVER AND DECAY

From: Mata et al.(2005) Trends Biochem Sci 30:506-514.



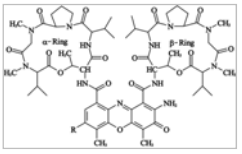


From: Mata et al.(2005) Trends Biochem Sci 30:506-514.

Can we measure RNA turn-over rate ?

How to measure mRNA half-life on single genes

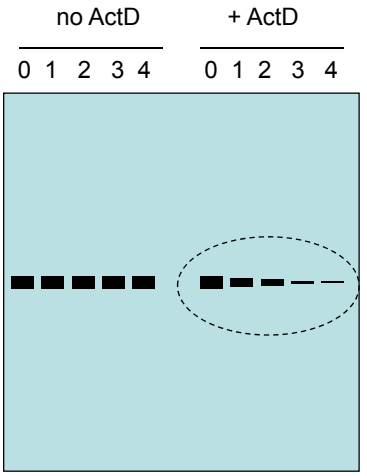
Cells treated with Actinomycin D



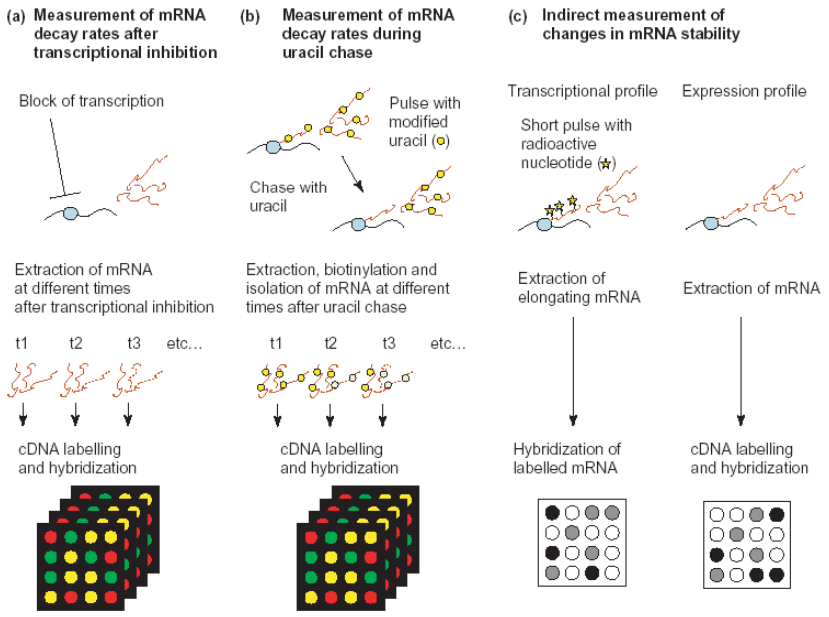
RNA extracted at time intervals

Add radioactive protection probe

RNase (ss)



RNase Protection Assay (RPA)



From: Mata et al.(2005) Trends Biochem Sci 30:506-514.

Letter

Decay Rates of Human mRNAs: Correlation With Functional Characteristics and Sequence Attributes

Edward Yang,[1,6] Erik van Nimwegen,[4,6] Mihaela Zavolan,[2] Nikolaus Rajewsky,[5] Mark Schroeder,[2] Marcelo Magnasco,[3] and James E. Darnell Jr.[1,7]

[1]Laboratory of Molecular Cell Biology, [2]Laboratory of Computational Genomics, [3]Laboratory of Mathematical Physics, and [4]Center for the Study of Physics and Biology, The Rockefeller University, New York, New York 10021-6399, USA; [5]Department of Biology and Courant Institute of Mathematical Sciences, New York University, New York, New York 10012, USA

Although mRNA decay rates are a key determinant of the steady-state concentration for any given mRNA species, relatively little is known, on a population level, about what factors influence turnover rates and how these rates are integrated into cellular decisions. We decided to measure mRNA decay rates in two human cell lines with high-density oligonucleotide arrays that enable the measurement of decay rates simultaneously for thousands of mRNA species. Using existing annotation and the Gene Ontology hierarchy of biological processes, we assign mRNAs to functional classes at various levels of resolution and compare the decay rate statistics between these classes. The results show statistically significant organizational principles in the variation of decay rates among functional classes. In particular, transcription factor mRNAs have increased average decay rates compared with other transcripts and are enriched in "fast-decaying" mRNAs with half-lives <2 h. In contrast, we find that mRNAs for biosynthetic proteins have decreased average decay rates and are deficient in fast-decaying mRNAs. Our analysis of data from a previously published study of *Saccharomyces cerevisiae* mRNA decay shows the same functional organization of decay rates, implying that it is a general organizational scheme for eukaryotes. Additionally, we investigated the dependence of decay rates on sequence composition, that is, the presence or absence of short mRNA motifs in various regions of the mRNA transcript. Our analysis recovers the positive correlation of mRNA decay with known AU-rich mRNA motifs, but we also uncover further short mRNA motifs that show statistically significant correlation with decay. However, we also note that none of these motifs are strong predictors of mRNA decay rate, indicating that the regulation of mRNA decay is more complex and may involve the cooperative binding of several RNA-binding proteins at different sites.

[Supplemental material is available online at www.genome.org, and also at http://genomes.rockefeller.edu/~yange.]

Actinomycin 2-3 hours - HepG2 cells
RNA extraction, labelling and → Affymetrix microarrays
Repeated on primary fibroblasts
Decay rates estimated for 5,245 genes.
Group of genes either short lived or long lived explored for ontology

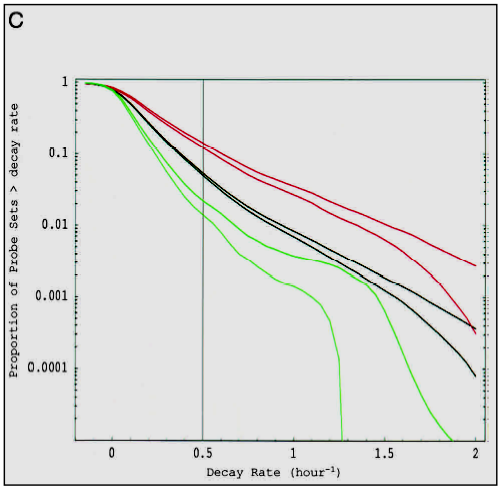
Figure 1 Functional analysis of decaying transcripts in human cells.

(C) Reverse cumulative distribution of decay rates for probe sets in different functional classes (HepG2 experiments). Decay rate r is shown horizontally, while vertically the fraction of probe sets with decay rates higher than r is plotted on a logarithmicsc ale. The pairs of lines show the 98% posterior probability intervals for the fraction at each value of r.

(Red) GO process transcription; (black) all probe sets;

(green) biosynthesis.

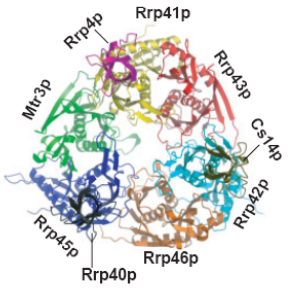
The gray line indicates the decay rate r = 0.5 h-1, which is our cutoff for fast decay in PFDI.

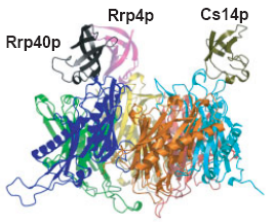


3'-5' degradation of the mRNA body: the exosome

is a large protein complex containing multiple 3'-5'-exonucleases

the core is composed of 9 subunits that form ringlike structures and use phosphate as an attacking group during RNA digestion, producing NDPs





Parker R. & Song H., 2004

- six of the nine core exosome subunit contain RNase PH domain

- three subunits show KH and S1 RNA-binding domains

- one additional exosome subunit is Rrp44, a 3' hydroxylase of RNaseD family

Regulating mRNA decay by *cis*-acting elements: ARE

- are A+U-rich elements found in the **3'-UTR** of some mRNAs encoding cytochines, proto-oncogenes and growth factors
- are defined by their ability to promote rapid deadenylation-dependent mRNA decay
- their sequence requirements are only loosely conserved

Group	Motif	Examples
I	WAUUUAW and a U-rich region	c-fos, c-myc
IIA	AUUUAUUUAUUUAUUUAUUUA	GM-CSF, TNF-α
IIB	AUUUAUUUAUUUAUUUA	Interferon-α
IIC	WAUUUAUUUAUUUAW	cox-2, IL-2, VEGF
IID	WWAUUUAUUUAWW	FGF2
IIE	WWWWAUUUAWWWW	u-PA receptor
III	U-rich, non-AUUUA	c-jun

Wilusz J.C. et al., 2001

- **ARE-binding proteins recognize these elements and, in conjunction with other proteins, will guide the mRNA to exosome degratation.**

Second class of *cis*-elements: target sites for microRNA (and other small ncRNAs)

Sequence elements in RNA are cis-acting and transferable

(see Luciferase reporter assay for RNA control elements in 3'UTR)

miRNA as mRNA regulators

- > 3000 miRNAs have been identified in vertebrates, flies, worms, plants and viruses
- 1000 or more miRNAs are predicted to function in humans, regulating 30% of human genes
- target mRNAs and biological function have been assigned to some miRNAs:
  - cell proliferation control (miR-181 and let-7)
  - hematopoietic B-cell lineage fate (miR-181)
  - B-cell survival (miR15a and miR16-1)
  - brain patterning (miR430)
  - pancreatic cell insulin secretion (miR375)
  - adipocyte development (miR-143)
  - .....

- expression of many miRNAs is specific to particular tissues or developmental stages
- deregulation of miRNA important in pathology (cancer, metabolic diseases)

In Cancer:

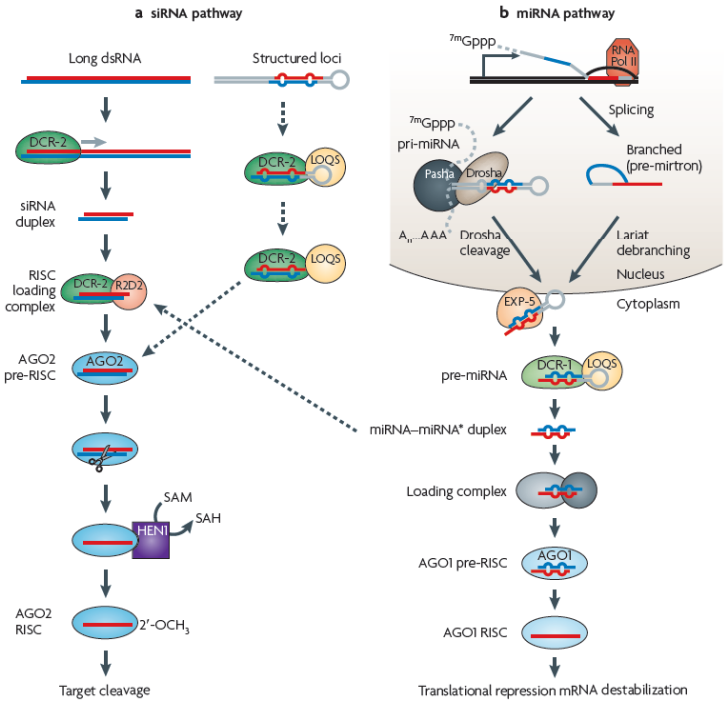
- altered patterns of miRNA expression may affect cell cycle and survival programs
- germ-line and somatic mutations in miRNAs and polymorphisms in the target mRNAs contribute to cancer predisposition and progression

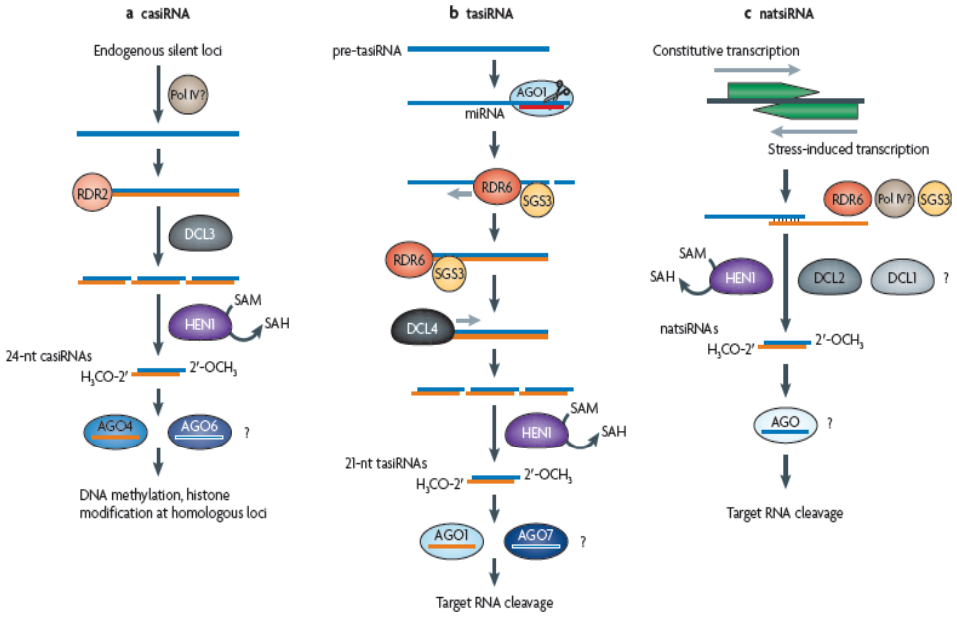
Small silencing RNAs: an expanding universe

Megha Ghildiyal and Phillip D. Zamore

Abstract | Since the discovery in 1993 of the first small silencing RNA, a dizzying number of small RNA classes have been identified, including microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs). These classes differ in their biogenesis, their modes of target regulation and in the biological pathways they regulate. There is a growing realization that, despite their differences, these distinct small RNA pathways are interconnected, and that small RNA pathways compete and collaborate as they regulate genes and protect the genome from external and internal threats.

review allegata a lezione 6





Cis-acting siRNAs (casiRNAs *TAS loci* *natural siRNA*

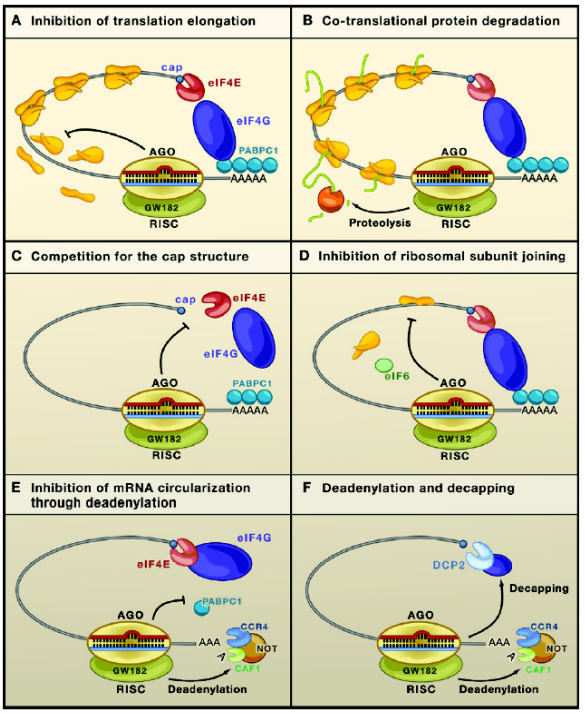


Figure 1. Mechanisms of miRNA-Mediated Gene Silencing

(A) Postinitiation mechanisms. MicroRNAs (miRNAs; red) repress translation of target mRNAs by blocking translation elongation or by promoting premature dissociation of ribosomes (ribosome drop-off).

(B) Cotranslational protein degradation. This model proposes that translation is not inhibited, but rather the nascent polypeptide chain is degraded cotranslationally. The putative protease is unknown.

(C–E) Initiation mechanisms. MicroRNAs interfere with a very early step of translation, prior to elongation. (C) Argonaute proteins compete with eIF4E for binding to the cap structure (cyan dot).

(D) Argonaute proteins recruit eIF6, which prevents the large ribosomal subunit from joining the small subunit.

(E) Argonaute proteins prevent the formation of the closed loop mRNA configuration by an ill-defined mechanism that includes deadenylation.

(F) MicroRNA-mediated mRNA decay. MicroRNAs trigger deadenylation and subsequent decapping of the mRNA target. Proteins required for this process are shown including components of the major deadenylase complex (CAF1, CCR4, and the NOT complex), the decapping enzyme DCP2, and several decapping activators (dark blue circles). (Note that mRNA decay could be an independent mechanism of silencing, or a consequence of translational repression, irrespective of whether repression occurs at the initiation or postinitiation levels of translation.) RISC is shown as a minimal complex including an Argonaute protein (yellow) and GW182 (green). The mRNA is represented in a closed loop configuration achieved through interactions between the cytoplasmic poly(A) binding protein (PABPC1; bound to the 3′ poly(A) tail) and eIF4G (bound to the cytoplasmic cap-binding protein eIF4E).

Determinants of miRNA/mRNA recognition: “seed” sequence

Minimum: positions 2-7 of the miRNA (from 5’) must Watson-Crick pair with mRNA

Seed sequence: 6-8 nt . Seeds longer than 6 nt may or not involve position 1.

Positions 10-11 should “bulge out” to guarantee miRNA functioning (Ago1 rather than Ago2)

Since the “seeding” sequence is very short (6-8nt), potential targets for known miRNA can be identified in the 3’-UTR of hundreds of genes each.

However, experiment using either trasfection of miRNA in cultured cells or knock-down of endogenous miRNA function by “antagomir” , followed by gene expression profiling with microarrays, demonstrated that a limited number of targets exist for each miRNA, and that (in fewer cases) new unidentified targets may exist.

Many studies have shown that several context-dependent factors are important :

1) the number of miRNA targets / 3’UTR
2) cooperativity with different miRNA
3) position of the targets
4) RNA-binding sites

To understand the proportion of protein synthesis inhibition *versus* mRNA degradation (cleavage), we should evaluate the **proteome** *versus* transcriptome

mRNA

•mRNAs represent a closer mirror of genome activity than proteins
•Homogeneous chemistry makes handling robust
•Complete knowledge of probes and control of hybridization conditions guarantees specificity

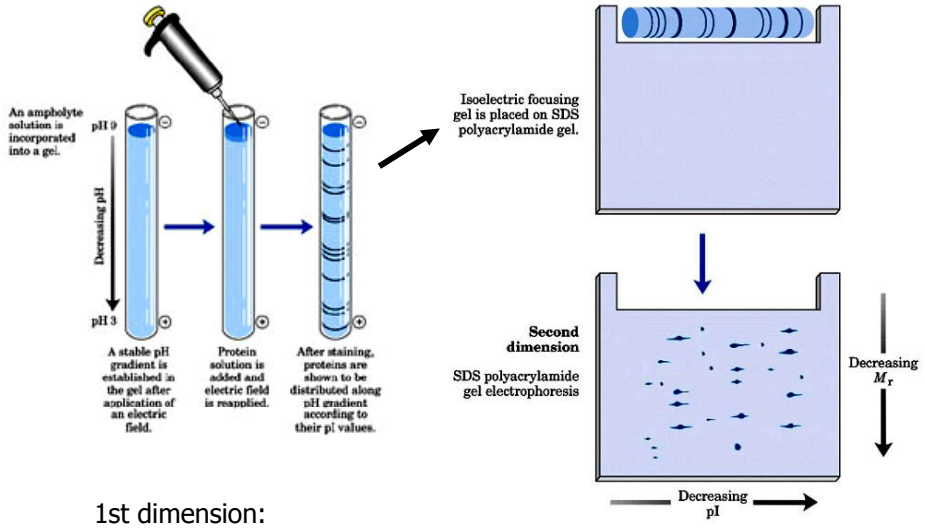
•The amount of any mRNA species is not necessarily proportional to the amount of the encoded protein
•One gene can encode for different proteins by alternative splicing

proteins

•Proteins are the real object of gene expression
•Separation and quantitation may be more reliable than for mRNAs

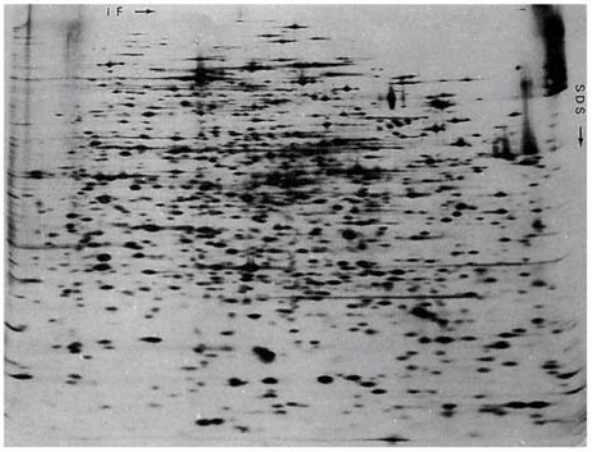
•Not all measured protein may represent "functional" protein.
•Dis-homogeneous chemistry makes it difficult to find procedures equally good for all proteins
•Specificity of interactions is hardly controlled, since it is based on a sum of different chemical interactions

Separation of complex protein mixtures by 2D gel electrophoresis



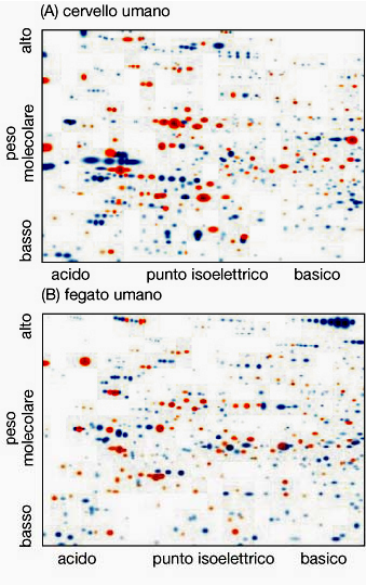
1st dimension: separation by isoelectric point

2nd dimension: separation by size



(b)

A 2D protein gel

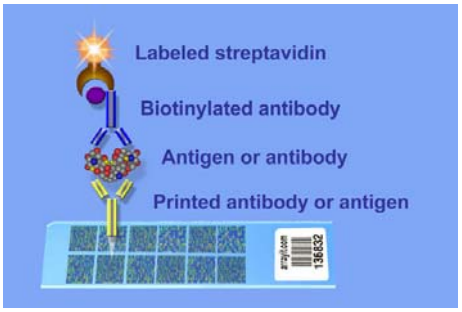


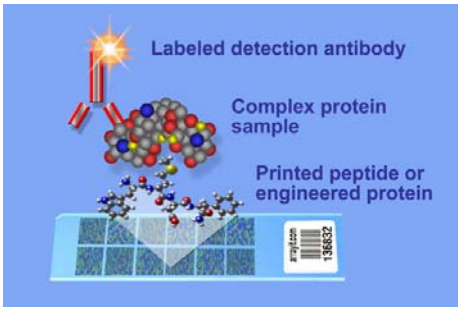
In red: common proteins

In blue: proteins expressed in either of the samples

Individual spots can be identified by mass spectrometry

Mass spectrometry identification of proteins





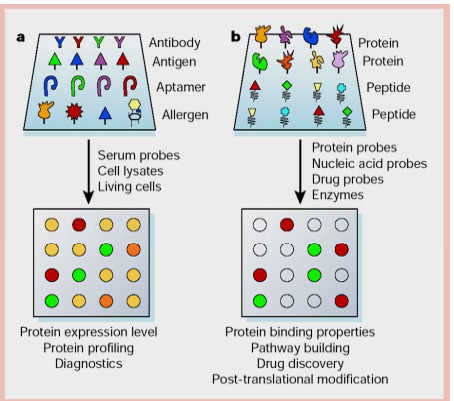


Figure 1 Analytical versus functional protein microarrays. **a**, Analytical protein microarray. Different types of ligands, including antibodies, antigens, DNA or RNA aptamers, carbohydrates or small molecules, with high affinity and specificity, are spotted down onto a derivatized surface. These chips can be used for monitoring protein expression level, protein profiling and clinical diagnostics. Similar to the procedure in DNA microarray experiments, protein samples from two biological states to be compared are separately labelled with red or green fluorescent dyes, mixed, and incubated with the chips. Spots in red or green colour identify an excess of proteins from one state over the other. **b**, Functional protein microarray. Native proteins or peptides are individually purified or synthesized using high-throughput approaches and arrayed onto a suitable surface to form the functional protein microarrays. These chips are used to analyse protein activities, binding properties and post-translational modifications. With the proper detection method, functional protein microarrays can be used to identify the substrates of enzymes of interest. Consequently, this class of chips is particularly useful in drug and drug-target identification and in building biological networks.

Applying mass spectrometry-based proteomics to genetics, genomics and network biology

Matthias Gstaiger[*‡] *and Ruedi Aebersold*[*‡§||]

Abstract | The systematic and quantitative molecular analysis of mutant organisms that has been pioneered by studies on mutant metabolomes and transcriptomes holds great promise for improving our understanding of how phenotypes emerge. Unfortunately, owing to the limitations of classical biochemical analysis, proteins have previously been excluded from such studies. Here we review how technical advances in mass spectrometry-based proteomics can be applied to measure changes in protein abundance, posttranslational modifications and protein–protein interactions in mutants at the scale of the proteome. We finally discuss examples that integrate proteomics data with genomic and phenomic information to build network-centred models, which provide a promising route for understanding how phenotypes emerge.

review

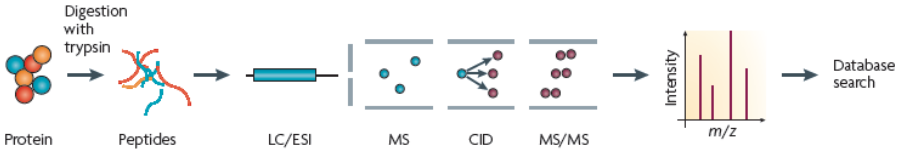


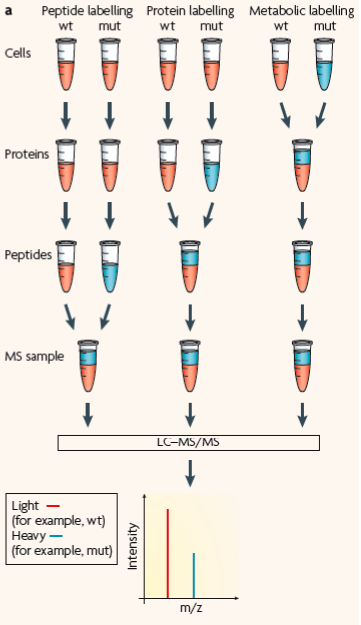
Figure 1 | **Mass spectrometry-based protein identification.** Protein samples are digested with trypsin and the resulting peptide mixtures are fractionated by reversed-phase liquid chromatography (LC). The fractionated peptide solution is subjected to an electric potential, which causes a spray to be formed, leading to the desolvation and ionization of the peptides (electrospray ionization; ESI). Mass to charge (*m*/*z*) ratios are measured from peptide ions that pass the collision cell without fragmentation in the mass spectrometer (MS). Specific ions are randomly selected for collision-induced dissociation (CID) with neutral gas molecules (for example, helium) and the resulting fragment ions are measured in the second mass analyser in tandem mass spectrometry (MS/MS). The MS precursor ion intensities obtained in the first stage can be used for peptide quantification, whereas MS/MS fragment ion information from the second stage contains sequence information that can be compared with sequences from *in silico* digested protein sequence databases for peptide and subsequent protein identification.

Isotope-labelling approaches

As shown in part **a** in the figure, differential labelling of proteins or peptides with heavy or light isotopes (indicated in red or blue) can be done *in vitro* or by the incorporation of isotope-labelled amino acids by metabolic labelling *in vivo*. For *in vitro* labelling, wild-type (wt) and mutant (mut) samples are prepared separately and isolated proteins or peptides are differentially labelled with heavy or light versions of isotope-tagging reagents, mainly through their sulphhydryl (for example, isotope-coded affinity tags)[71] or amine groups (for example, isotope-coded protein labels)[73]. Differential labelling introduces a characteristic mass shift, which can be used to determine the MS1 peptide ratios between pairs of heavy and light peptides. Peptide labelling with recently introduced isobaric tags for relative and absolute quantitation, which as the name indicates, keep the mass of the differentially labelled precursor ions of a given peptide constant but allow quantification after tandem mass spectrometry (MS/MS) analysis on the basis of sample-specific reporter ion intensities from up to eight different samples in a single liquid chromatography–tandem mass spectrometry (LC–MS/MS) experiment[74,75].

The use of synthetic isotope-labelled reference peptides for absolute quantification that was pioneered by Desiderio *et al.*[76] has been extended to proteomic studies by Steve Gygi and colleagues[77]. In this approach, known amounts of synthetic isotope-labelled reference peptides, which correspond to proteotypic peptides of the proteins to be analysed, are added to the samples before LC–MS/MS analysis for absolute quantification of proteins.

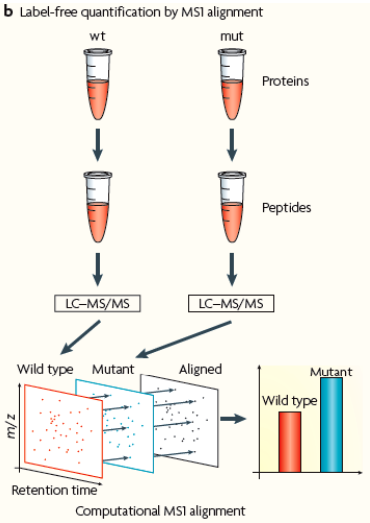
Stable isotope labelling with amino acids in cell culture is an *in vivo* isotope labelling method that is becoming increasingly popular[78]. Wild-type and mutant cells are grown in media that contains either light or heavy isotope versions of lysine or arginine, which yield differentially labelled proteomes. The entire labelling process occurs



at the beginning of the experiment, which has the advantage that samples can be combined at early steps to avoid errors that can be introduced when samples are separately processed. As the method is limited to cells or organisms that can be metabolically labelled, it is not generally applicable to human tissues and body fluids.

Label-free quantification from aligned MS1 spectra

For label-free quantification (part **b** in the figure) wild-type and mutant proteomes are analysed by separate LC–MS/MS experiments and the MS1 spectra are computationally aligned to calculate the relative protein abundance changes on the basis of the signal intensities of extracted ion chromatograms from aligned peptide features. This reduces the undersampling problem that is known to occur with MS/MS-based approaches and results in a dynamic range of three to four orders of magnitude[79]. Newer hybrid MS instruments (LTQ FT and LTQ Orbitrap) offer the option to simultaneously record MS signal intensities and identify peptides using MS/MS. These two types of information can be combined by recently developed computational approaches[80–82]. The number of peptides that can be mapped across different LC–MS/MS experiments therefore depends on the accuracy of the peptide masses that are determined by the mass analyser and reproducibility of the LC system. Strategies for signal normalization and for correcting variations in LC performances have been developed and are now integrated in automated computational platforms for label-free MS analysis[83].



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ARTICLES

Widespread changes in protein synthesis induced by microRNAs

Matthias Selbach[1], Björn Schwanhäusser[1]*, Nadine Thierfelder[1]*, Zhuo Fang[1], Raya Khanin[2] & Nikolaus Rajewsky[1]

Animal microRNAs (miRNAs) regulate gene expression by inhibiting translation and/or by inducing degradation of target messenger RNAs. It is unknown how much translational control is exerted by miRNAs on a genome-wide scale. We used a new proteomic approach to measure changes in synthesis of several thousand proteins in response to miRNA transfection or endogenous miRNA knockdown. In parallel, we quantified mRNA levels using microarrays. Here we show that a single miRNA can repress the production of hundreds of proteins, but that this repression is typically relatively mild. A number of known features of the miRNA-binding site such as the seed sequence also govern repression of human protein synthesis, and we report additional target sequence characteristics. We demonstrate that, in addition to downregulating mRNA levels, miRNAs also directly repress translation of hundreds of genes. Finally, our data suggest that a miRNA can, by direct or indirect effects, tune protein synthesis from thousands of genes.

Article