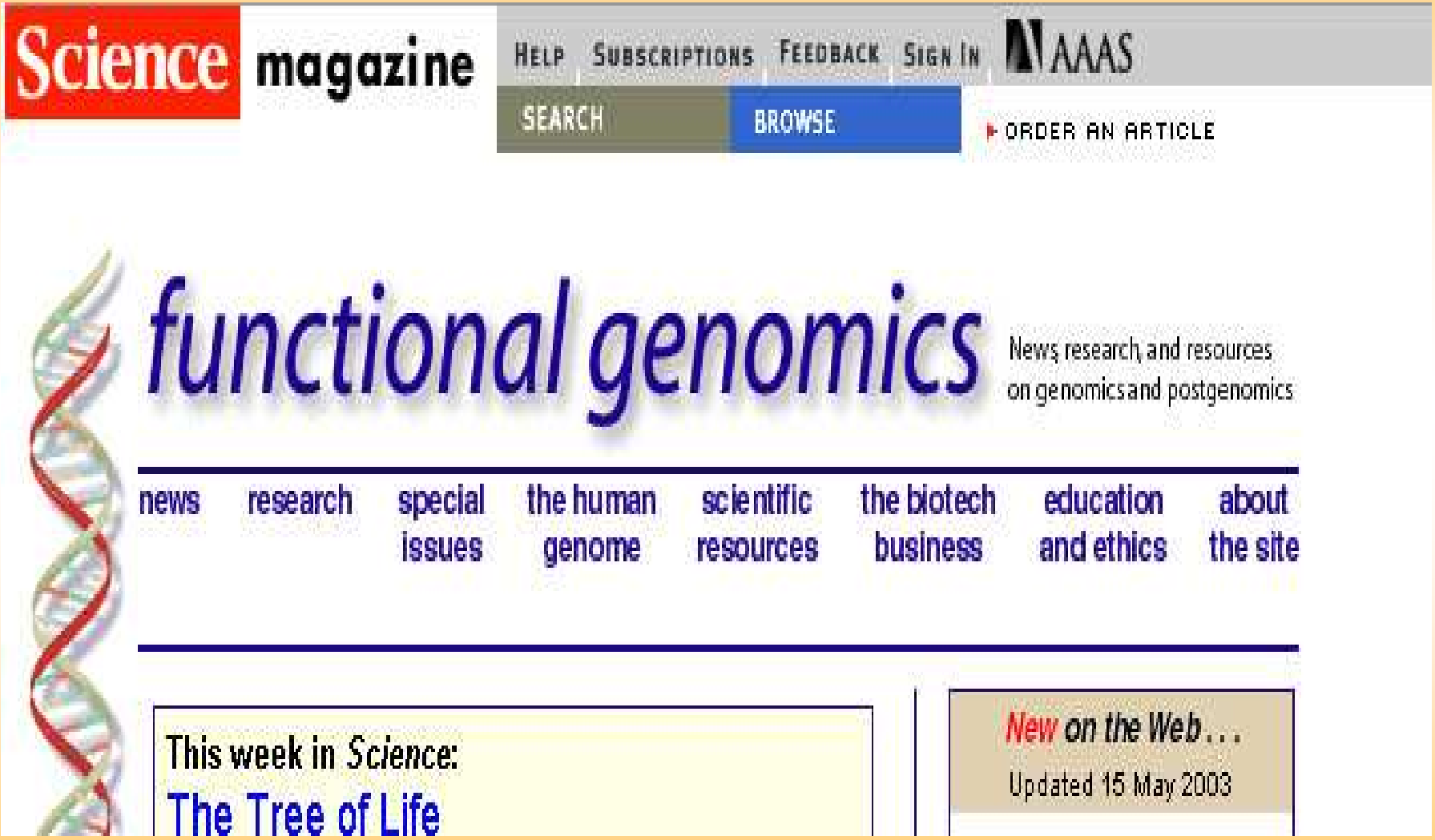
Genomica Funzionale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functional genomics
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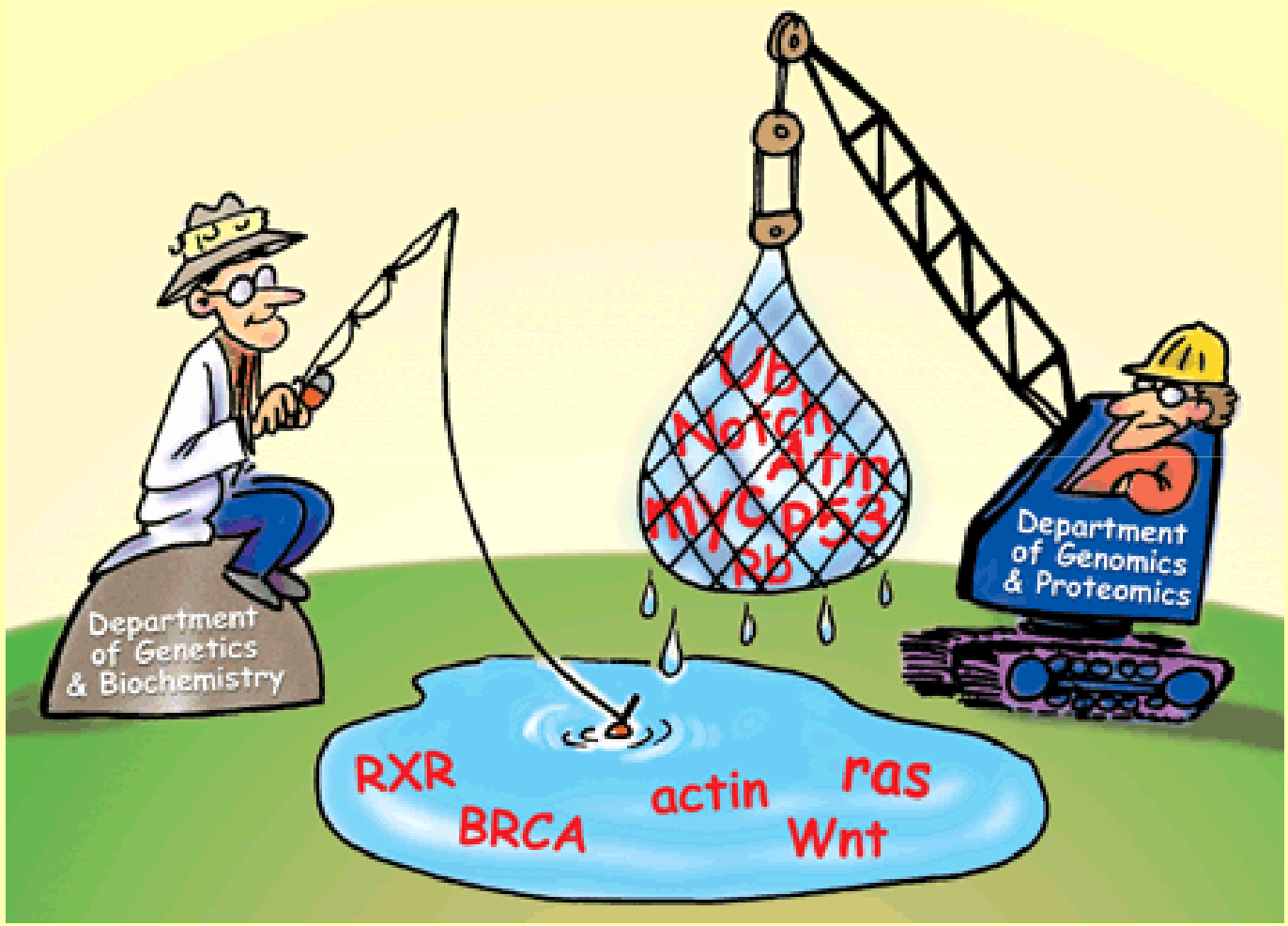
Updated 15 May 2003

- Genomics: History, Future, Whole Genomes and Comparative Genomics
- Gene Function and Expression
  - Global Gene Expression
- Relating Sequence to Structure to Function
- Molecular Evolution
- Animal Models
- Genetically Modified Plants
- Proteomics
- Genome Organization and Chromosome Dynamics
- Gene and Genome Technologies
  - Sequencing Technologies
  - Gene Targeting
  - Applications of Genome Information
- Disease-Associated Genes
- Gene Therapy
- Genetic Testing
- DNA Vaccines

Genomica funzionale



Functional Genomics

- Biochemical Genomics
- Biophysical Genomics
- Physiological Genomics
- Cell Genomics
- ……

Functional Genomics
Levels

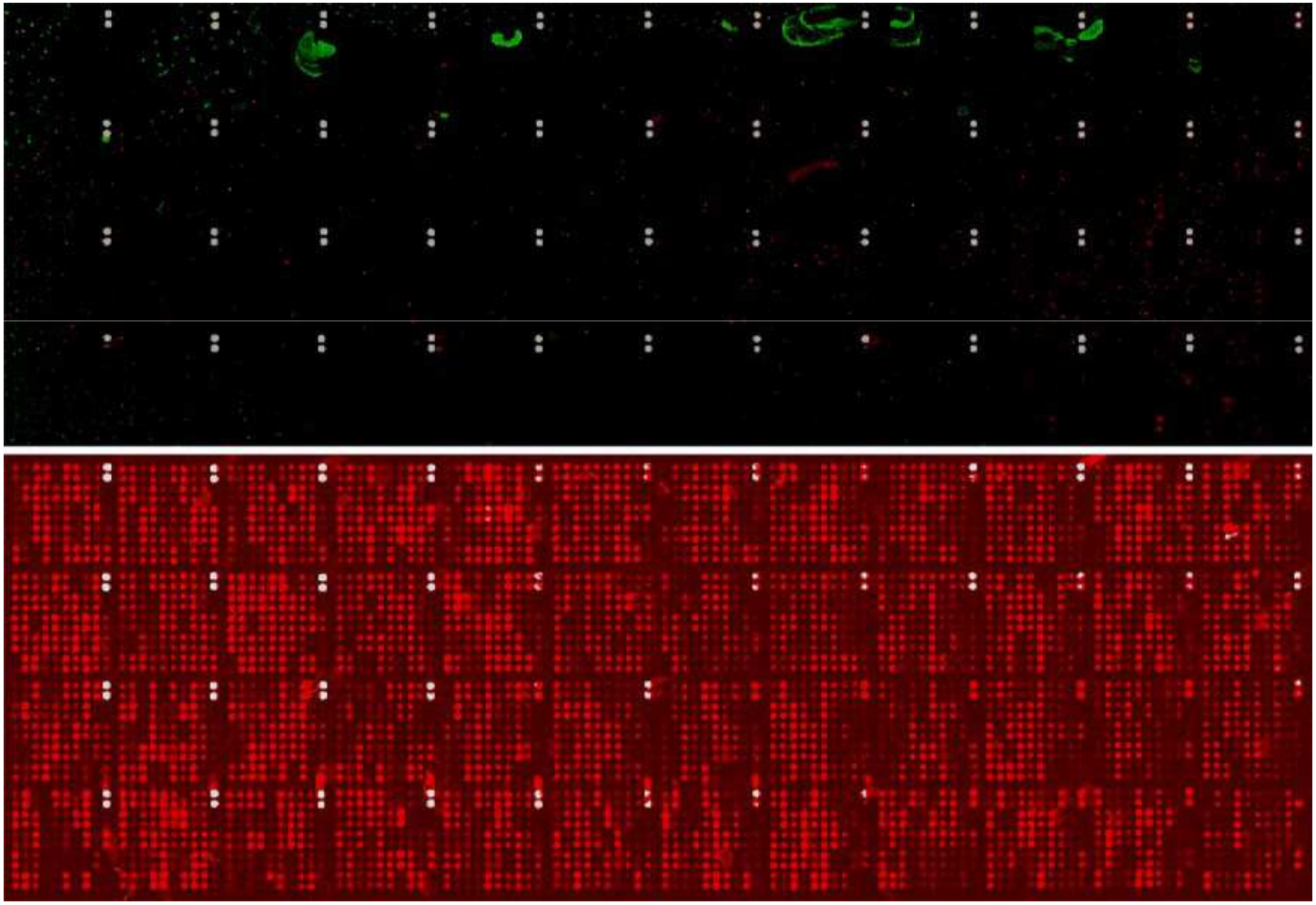
- ✓ Genome to transcriptome
- ❑ Transcriptome to proteome
- ❑ Proteome to dynamic system
- ❑ Dynamic systems to phenotype

Proteome investigation

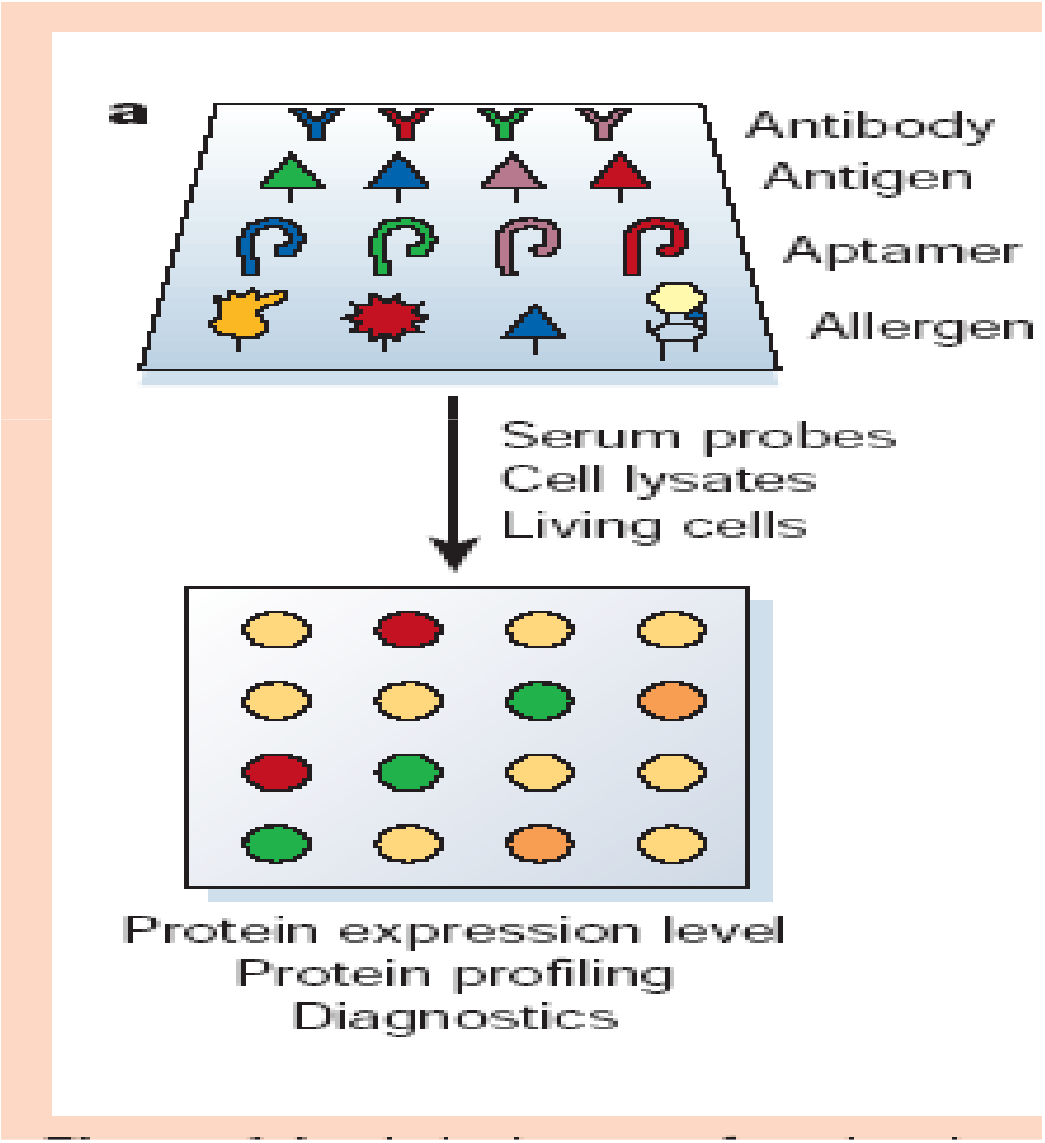
- 2D Gels
- Arrays

Protein analysis on a proteomic scale

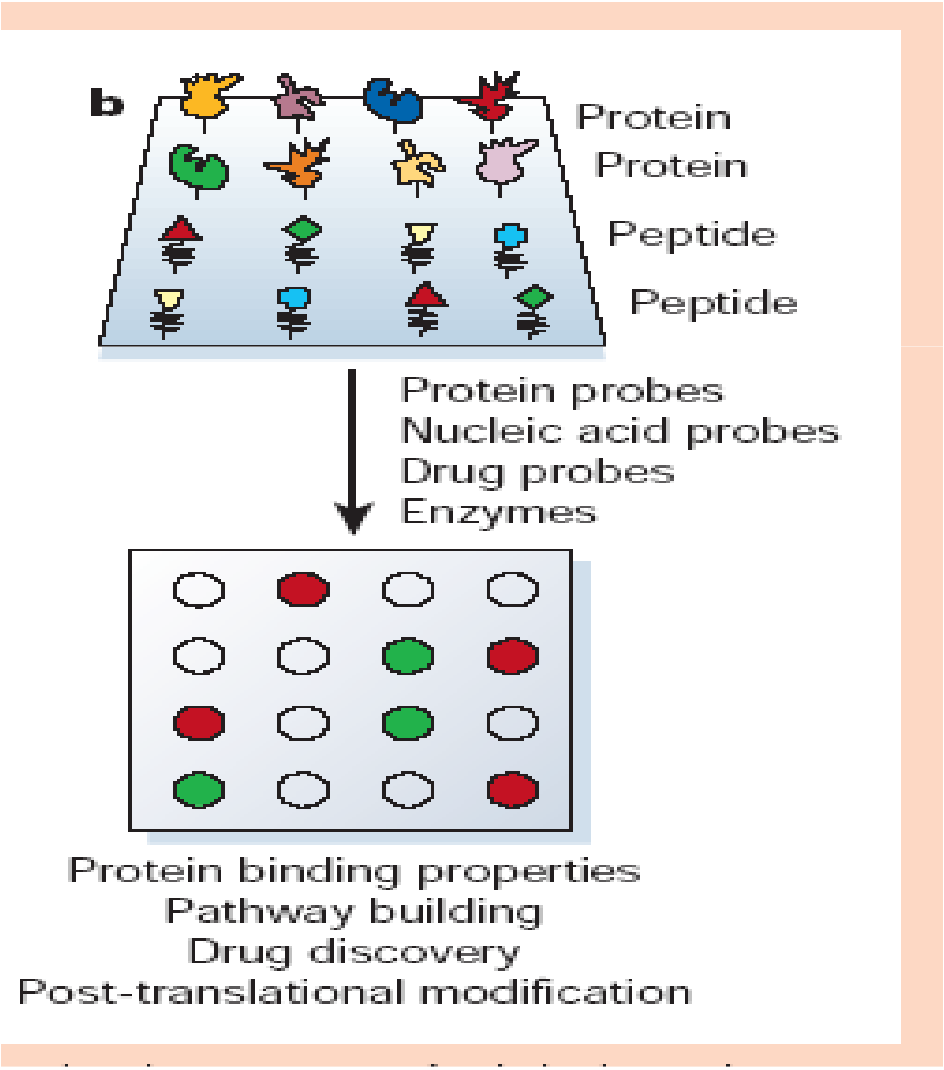
ARRAYS



Protein analysis on a proteomic scale
Analytical



Protein analysis on a proteomic scale
Functional



Protein analysis on a proteomic scale

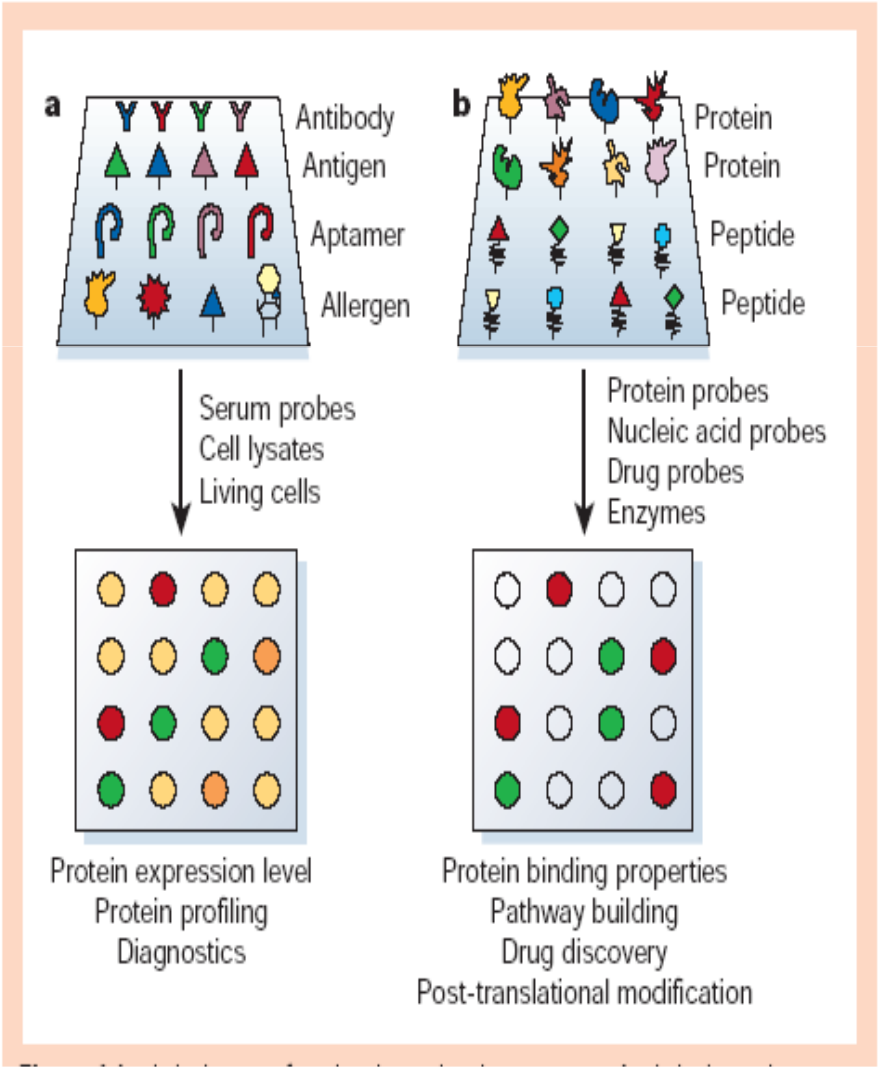
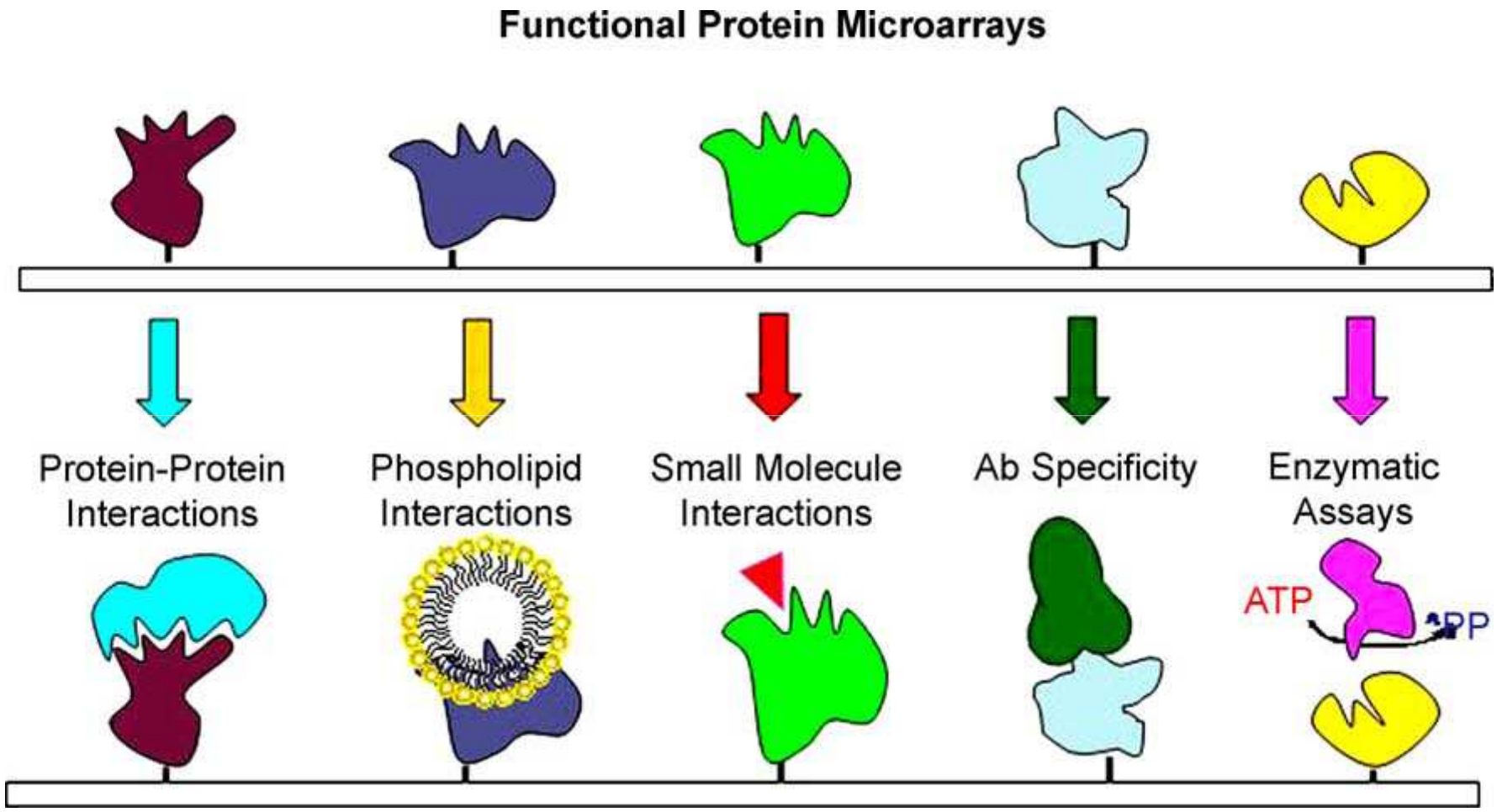
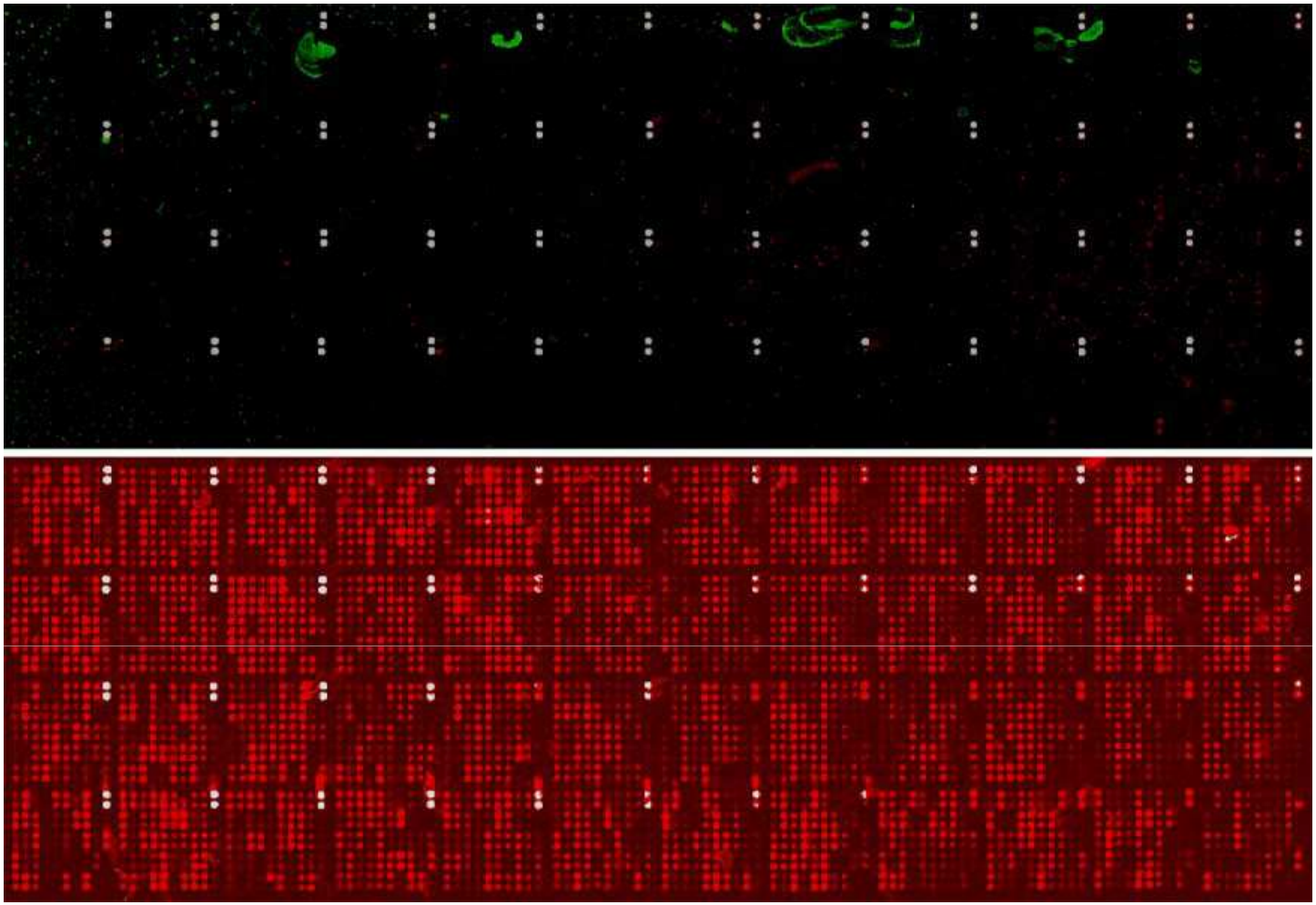


Figure 1 Analytical versus functional protein microarrays. **a**, Analytical protein microarray. Different types of ligands, including antibodies, antigens, DNA or RNA aptamers, carbohydrates or small molecules, with high affinity and specificity, are spotted down onto a derivatized surface. These chips 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.

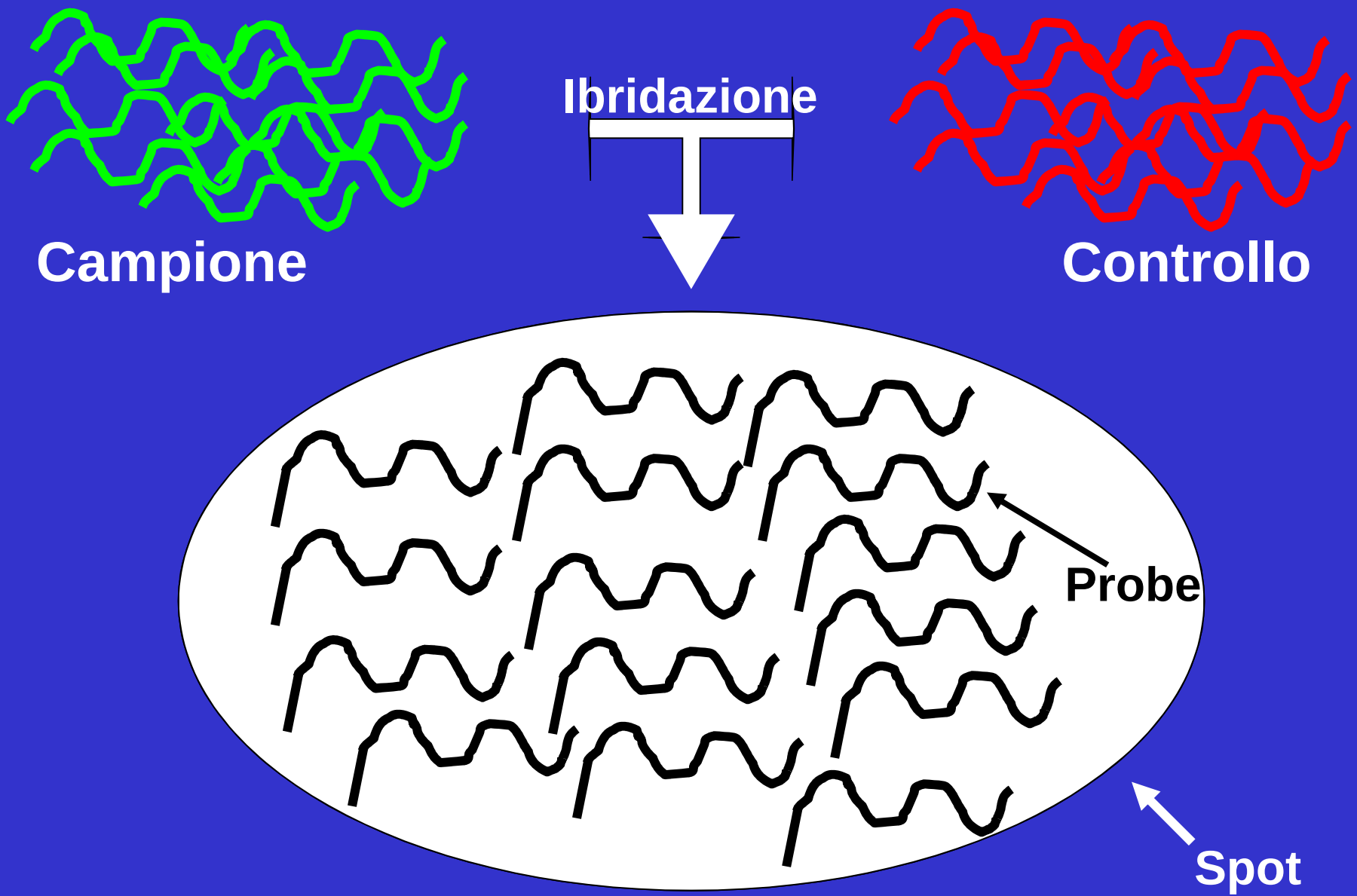
Protein analysis on a proteomic scale



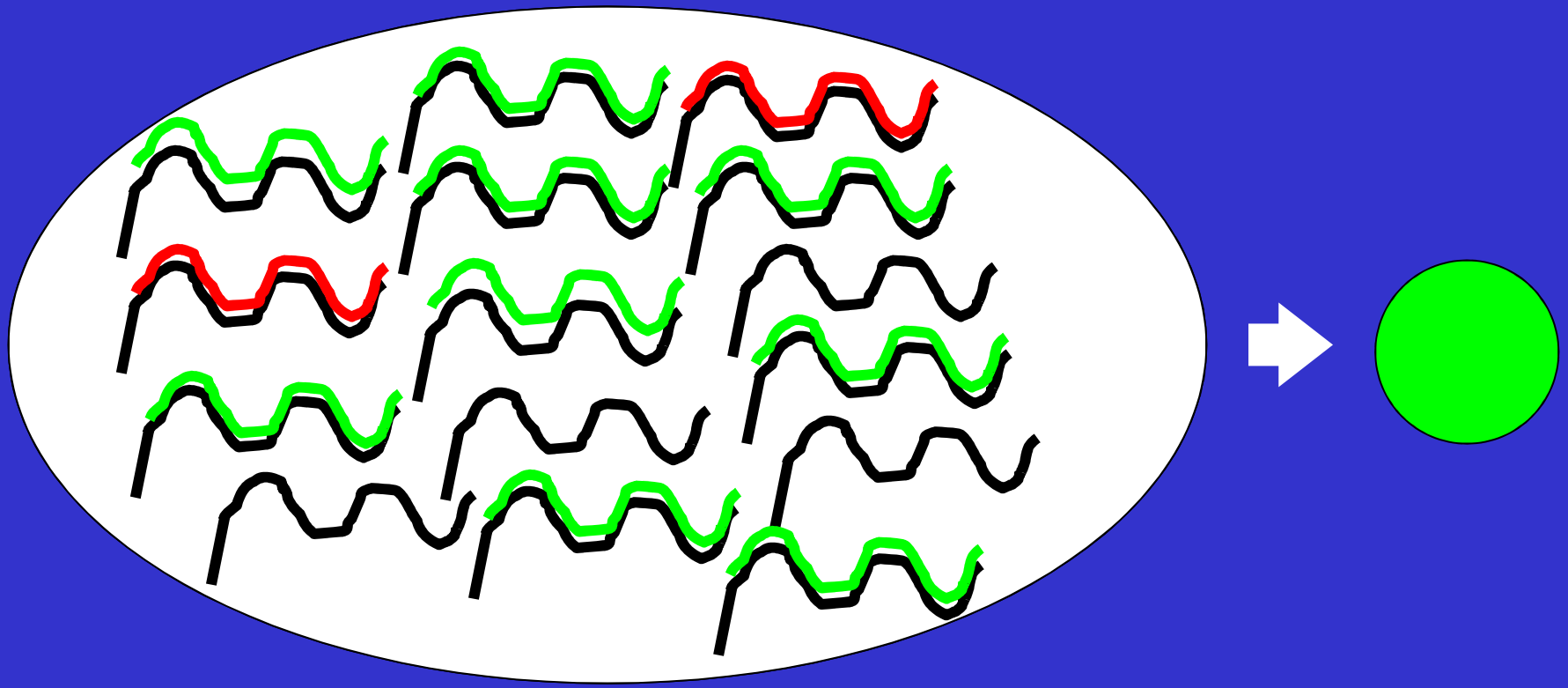


Yeast protein microarray. RED , a yeast protein microarray probed with anti-GST antibody followed by probing with Cy5-conjugated anti-rabbit antibody. The relative level of the printed GST-fused proteins can be determined.GREEN, the same microarray probed Cy3-conjugated streptavidin, which recognizes the biotinylated control proteins printed on the slide.

3) Ibridazione e lettura del microarray

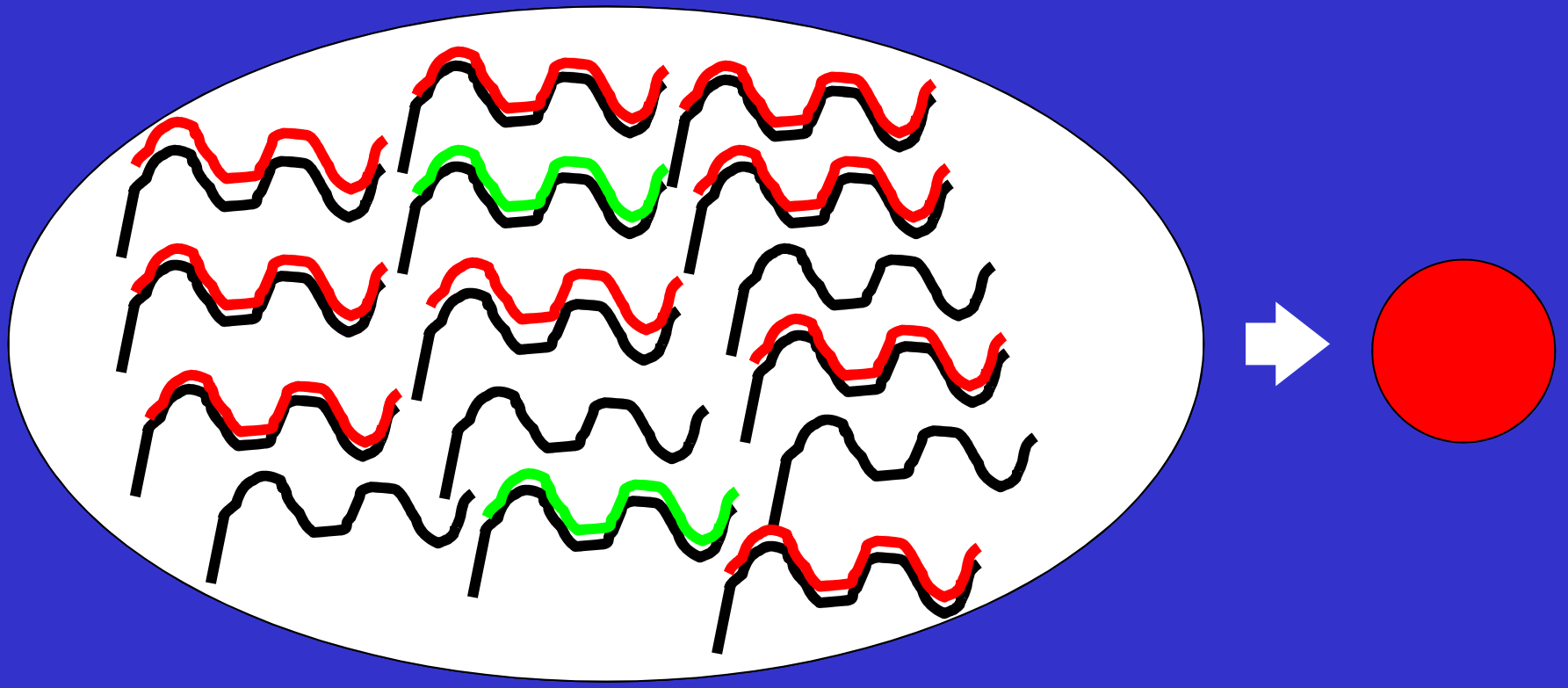


3) Ibridazione e lettura del microarray



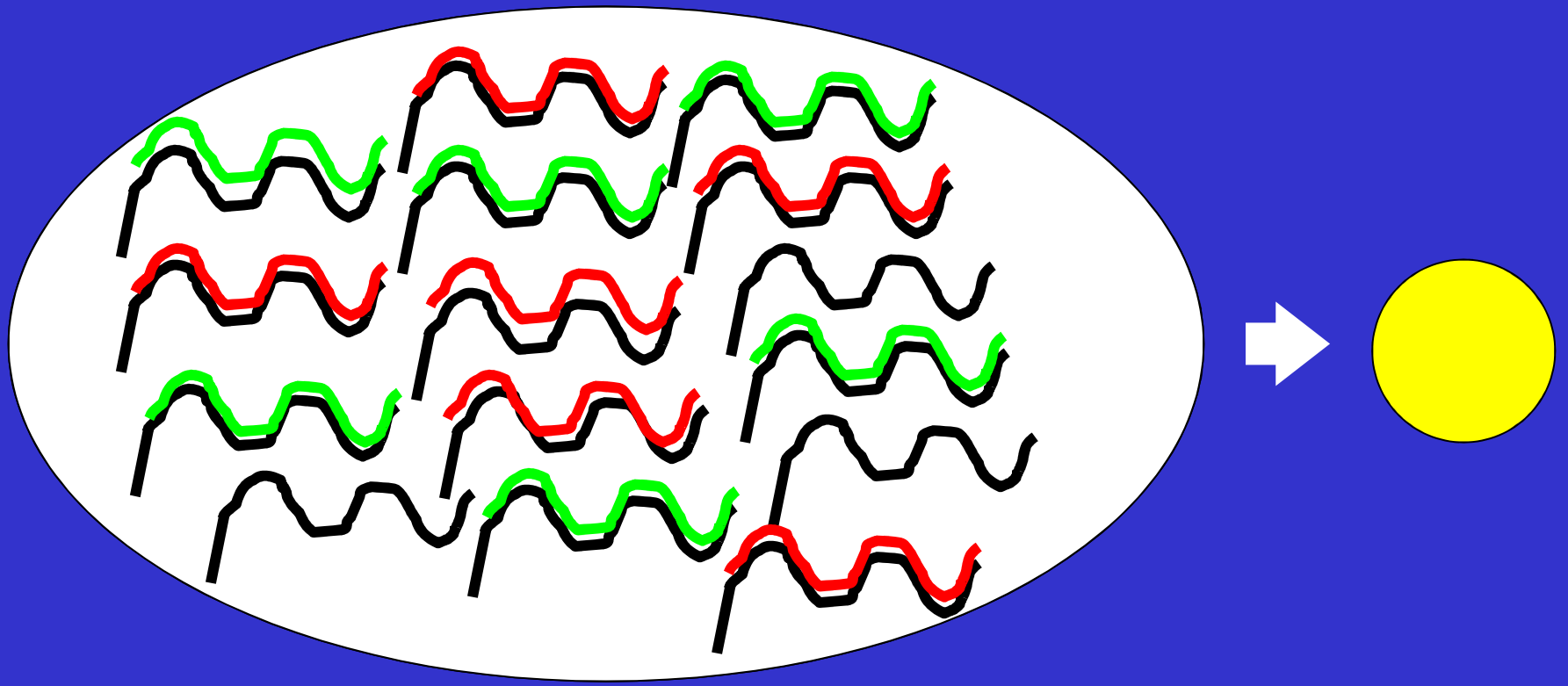
Il gene è espresso a livelli più alti nel **campione** (**più** copie del mRNA prodotte) rispetto al **controllo** quindi prevale la fluorescenza “verde”

3) Ibridazione e lettura del microarray



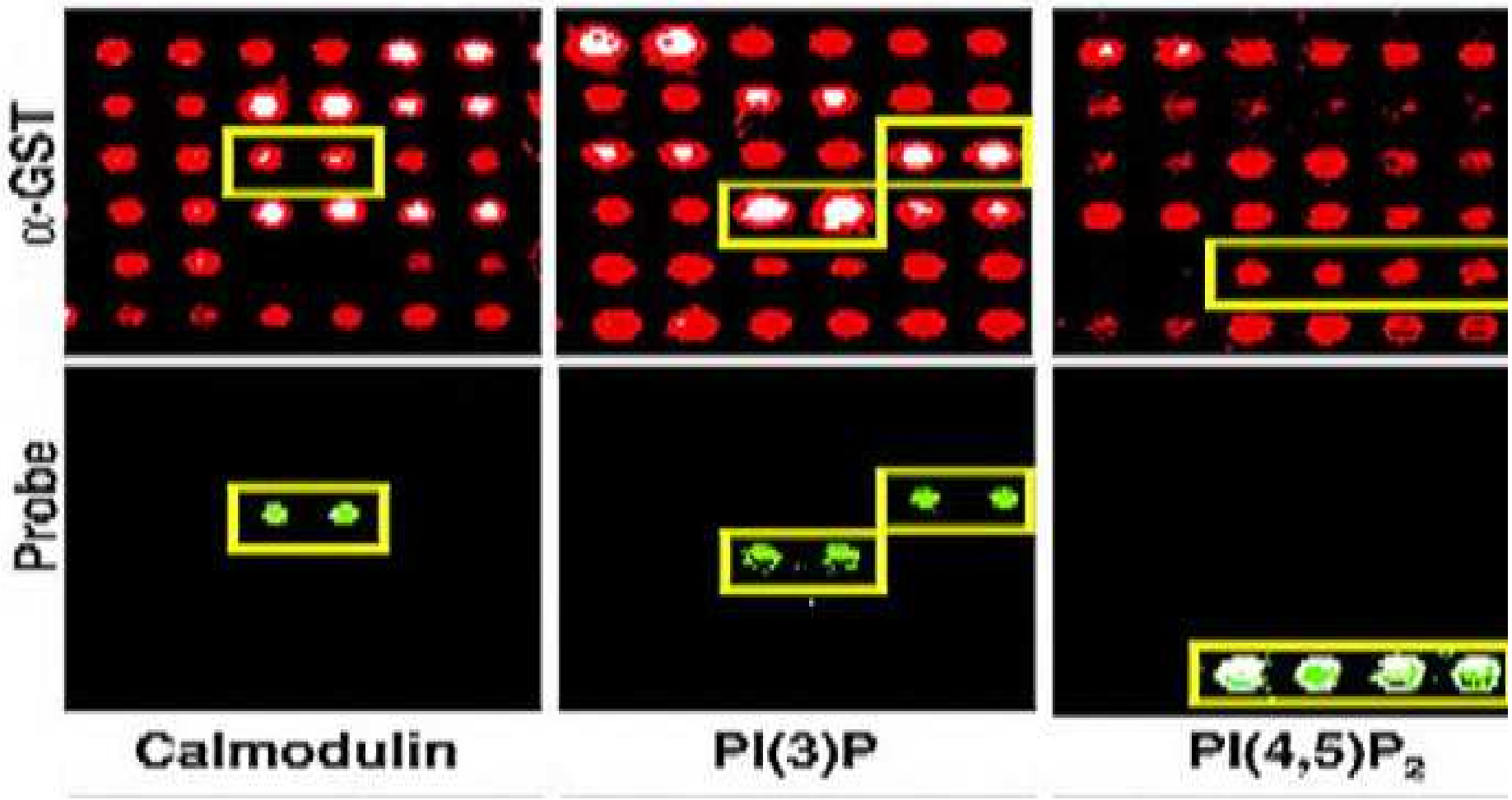
Il gene è espresso a livelli più bassi (**meno** copie del mRNA prodotte) nel **<u>campione</u>** rispetto al **<u>controllo</u>** quindi prevale la fluorescenza “rossa”

3) Ibridazione e lettura del microarray



Il gene è espresso a livelli simili in campioni testati, quindi non prevale nessuna delle due fluorescenze

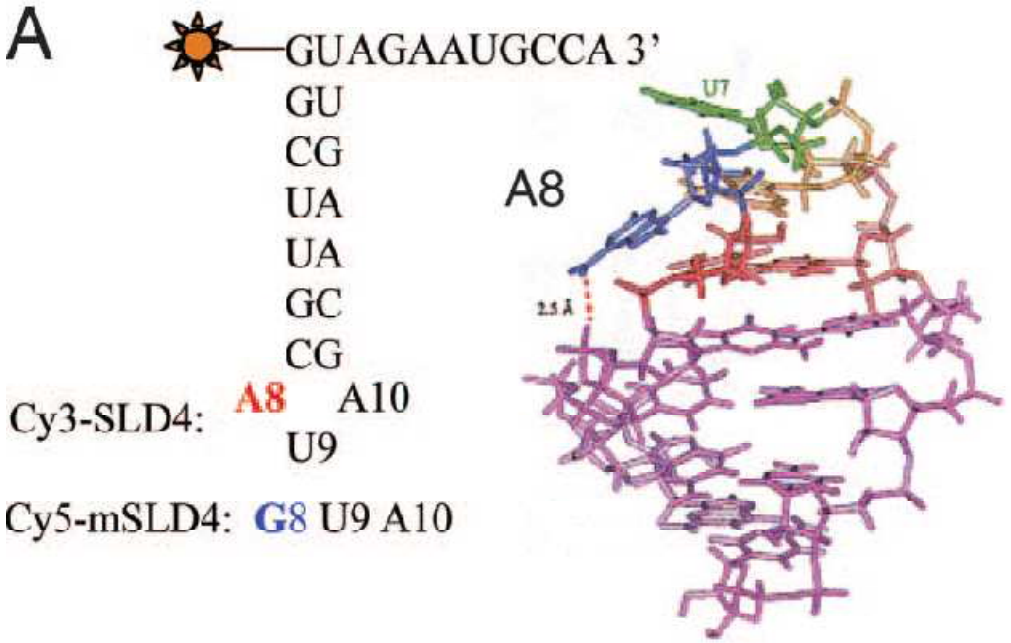
Proteome: Global Analysis of Protein Activities Using Proteome Chips



Zhu et al 2001

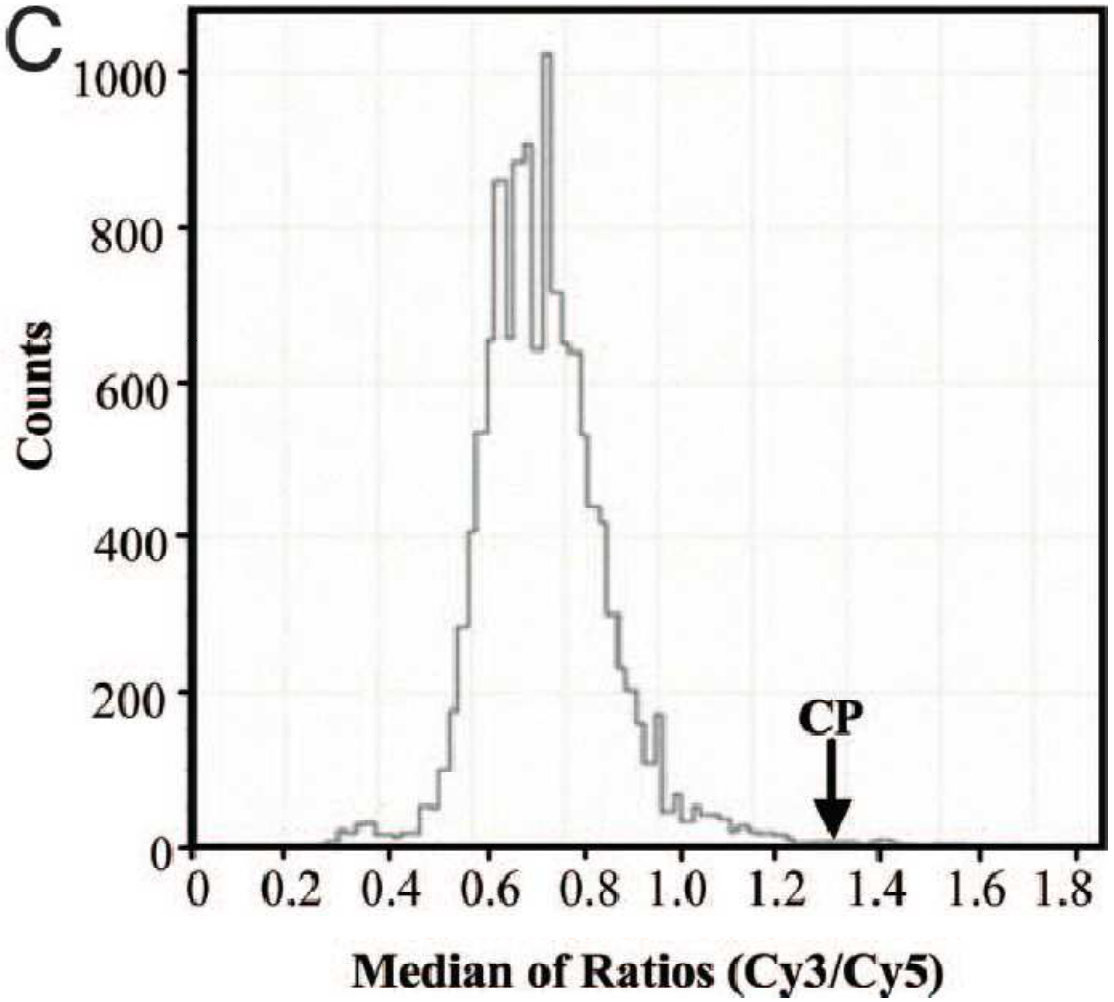
- To facilitate studies of the yeast proteome, we cloned 5800 open reading frames and overexpressed and purified their corresponding proteins. The proteins were printed onto slides at high spatial density to form a yeast proteome microarray and screened for their ability to interact with proteins and phospholipids. We identified many new calmodulin- and phospholipid-interacting proteins; a common potential binding motif was identified for many of the calmodulin-binding proteins. Thus, microarrays of an entire eukaryotic proteome can be prepared and screened for diverse biochemical activities. The microarrays can also be used to screen protein-drug interactions and to detect posttranslational modifications.

Probe: RNA-binding proteins

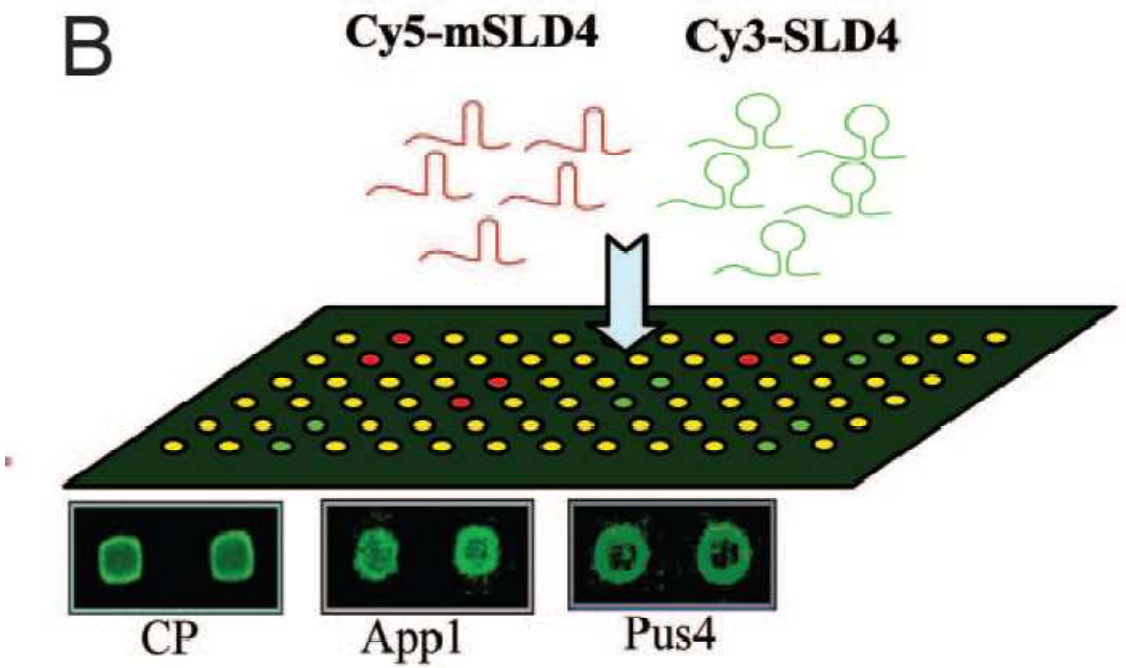


small RNA hairpin that contains a clamped adenine (A8) motif (CAM).
A CAM is required for the replication of Brome Mosaic Virus (BMV).

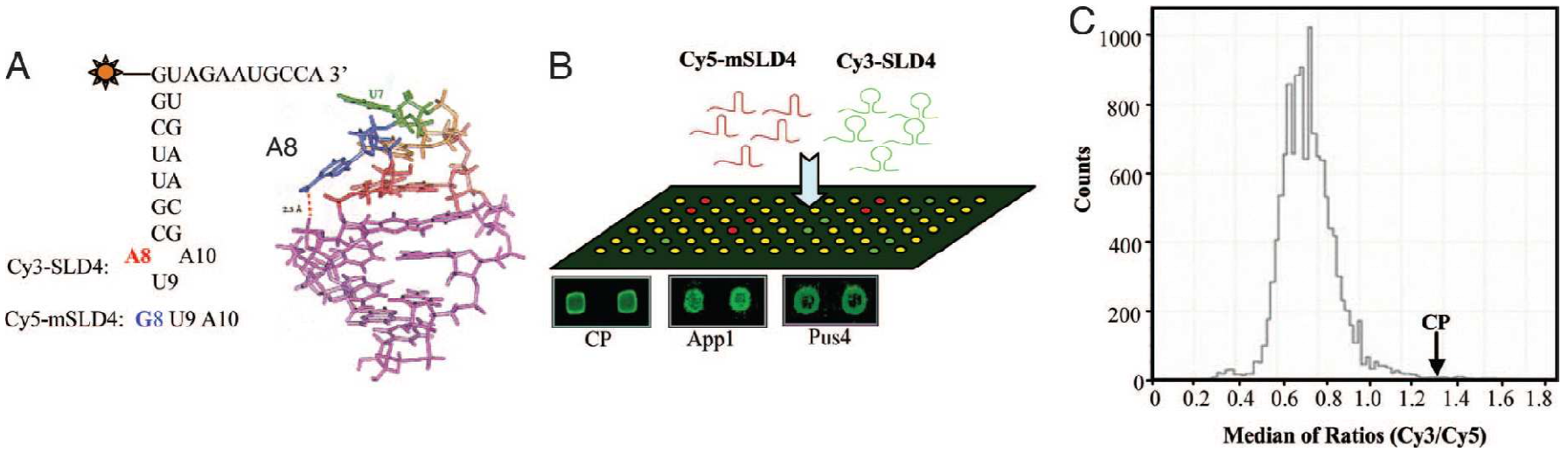
RNA-binding proteins



RNA-binding proteins



RNA-binding proteins



- **Pseudouridine Synthase 4 (Pus4) and the Actin Patch Protein 1 (App1)**
- **modestly reduced BMV genomic plus-strand RNA accumulation**
- **dramatically inhibited BMV systemic spread in plants.**
- **Pus4 also prevented the encapsidation of a BMV RNA in plants and the reassembly of BMV virions *in vitro*.**

- **These results demonstrate the feasibility of using proteome arrays to identify specific RNAbinding proteins for antiviral activities**

- **Arrays of>5,000 *Saccharomyces cerevisiae* proteins were screened to identify proteins that can preferentially bind a small RNA hairpin that contains a clamped adenine motif (CAM).**
- **A CAM is required for the replication of Brome Mosaic Virus (BMV), a plant-infecting RNA virus that can replicate in *S. cerevisiae*.**
- **Several hits were selected for further characterization in *Nicotiana benthamiana*.**
- **Pseudouridine Synthase 4 (Pus4) and the Actin Patch Protein 1 (App1) modestly reduced BMV genomic plus-strand RNA accumulation, but dramatically inhibited BMV systemic spread in plants.**
- **Pus4 also prevented the encapsidation of a BMV RNA in plants and the reassembly of BMV virions *in vitro*.**
- **These results demonstrate the feasibility of using proteome arrays to identify specific RNAbinding proteins for antiviral activities.**
- **Furthermore, the effects of Pus4 suggest that the CAM-containing RNA motif provides a regulatory link between RNA replication and encapsidation**

❖**Microarray:**

282 known and potential yeast transcription factors

❖**Probes :**

oligonucleotides of evolutionarily conserved sequences that are potentially functional.

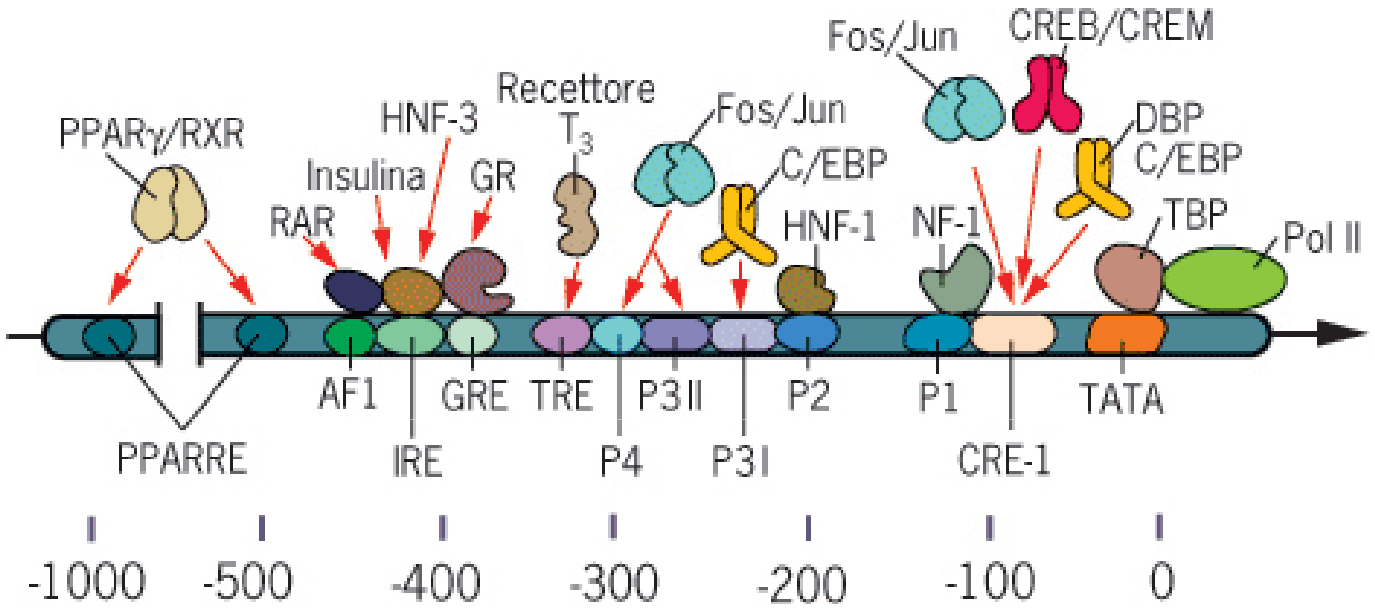
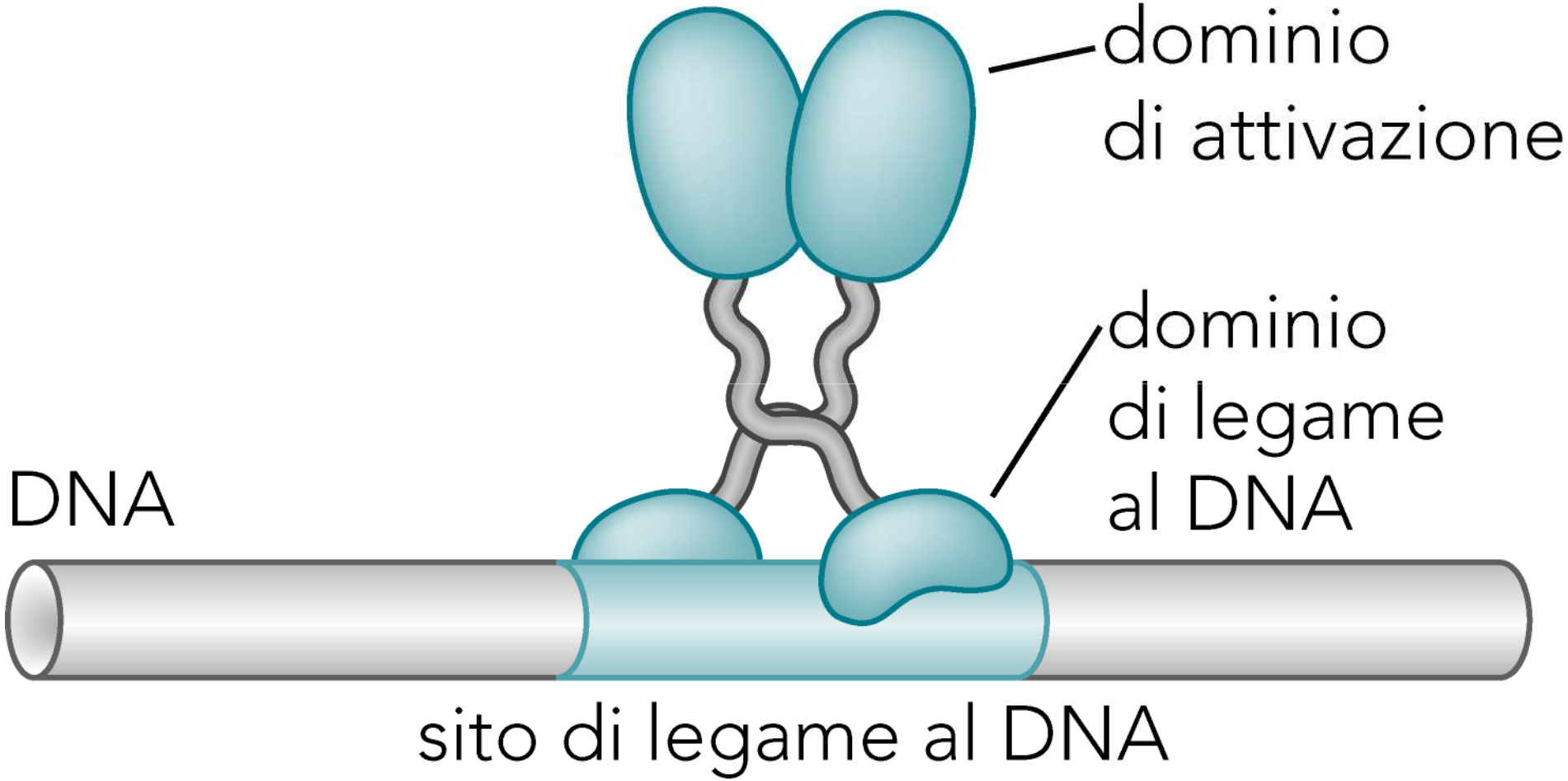
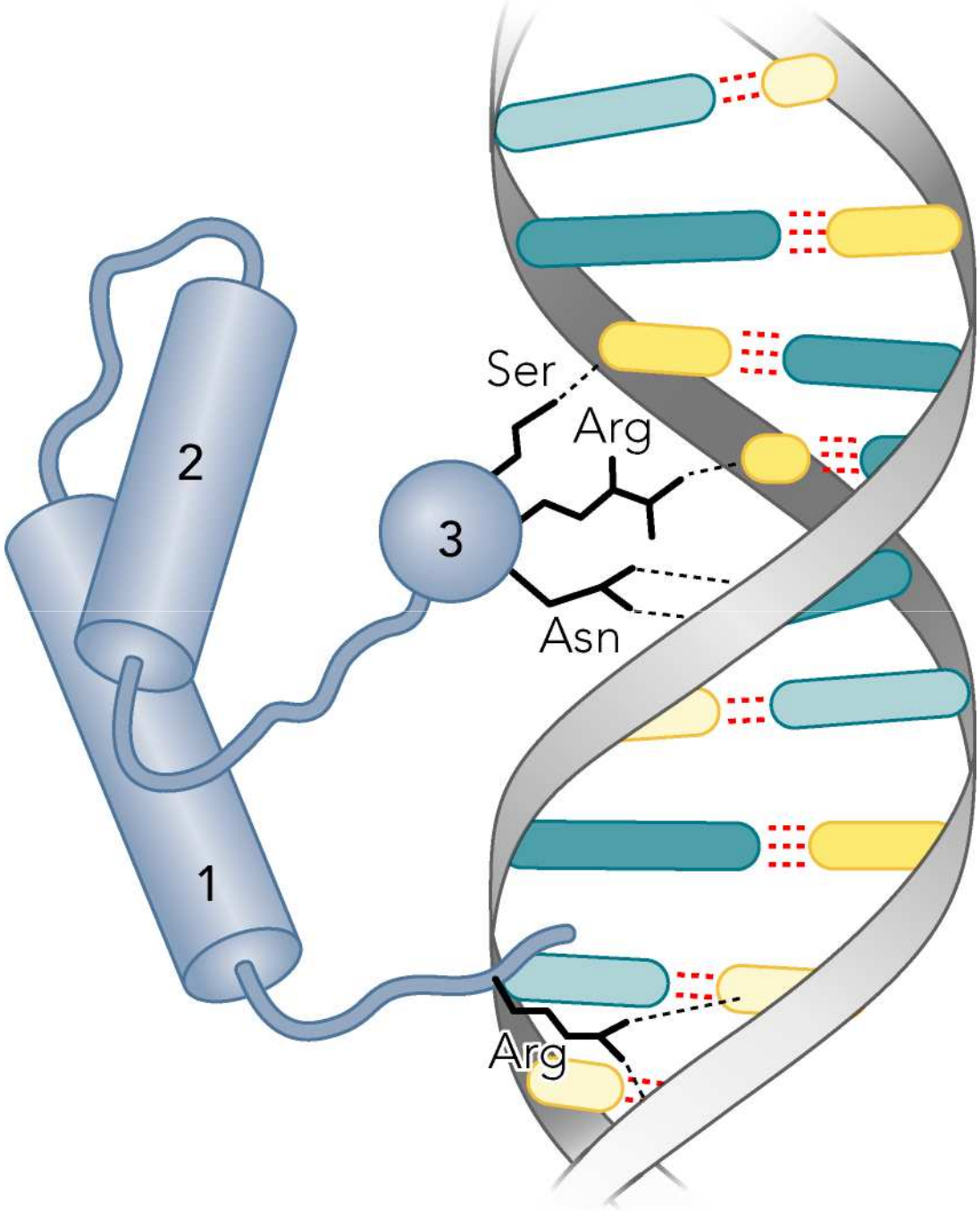


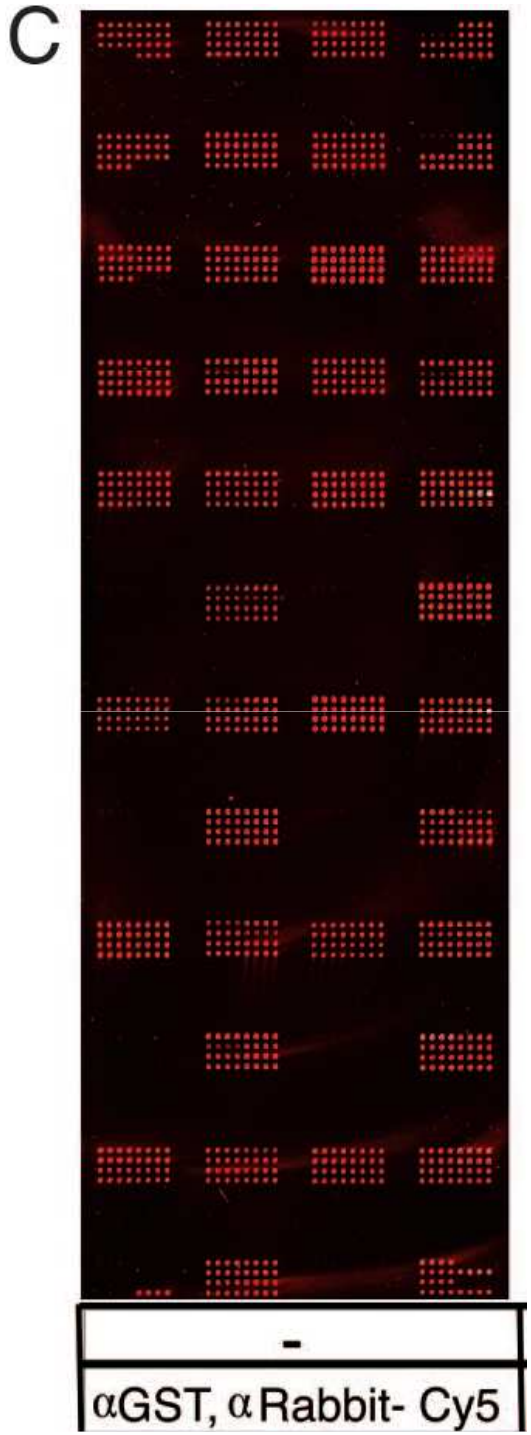
FIGURA 12.43 **Regolazione dela trascrizione del gene *PEPCK* di ratto.** La trascrizione di questo gene, come negli altri casi, è con-

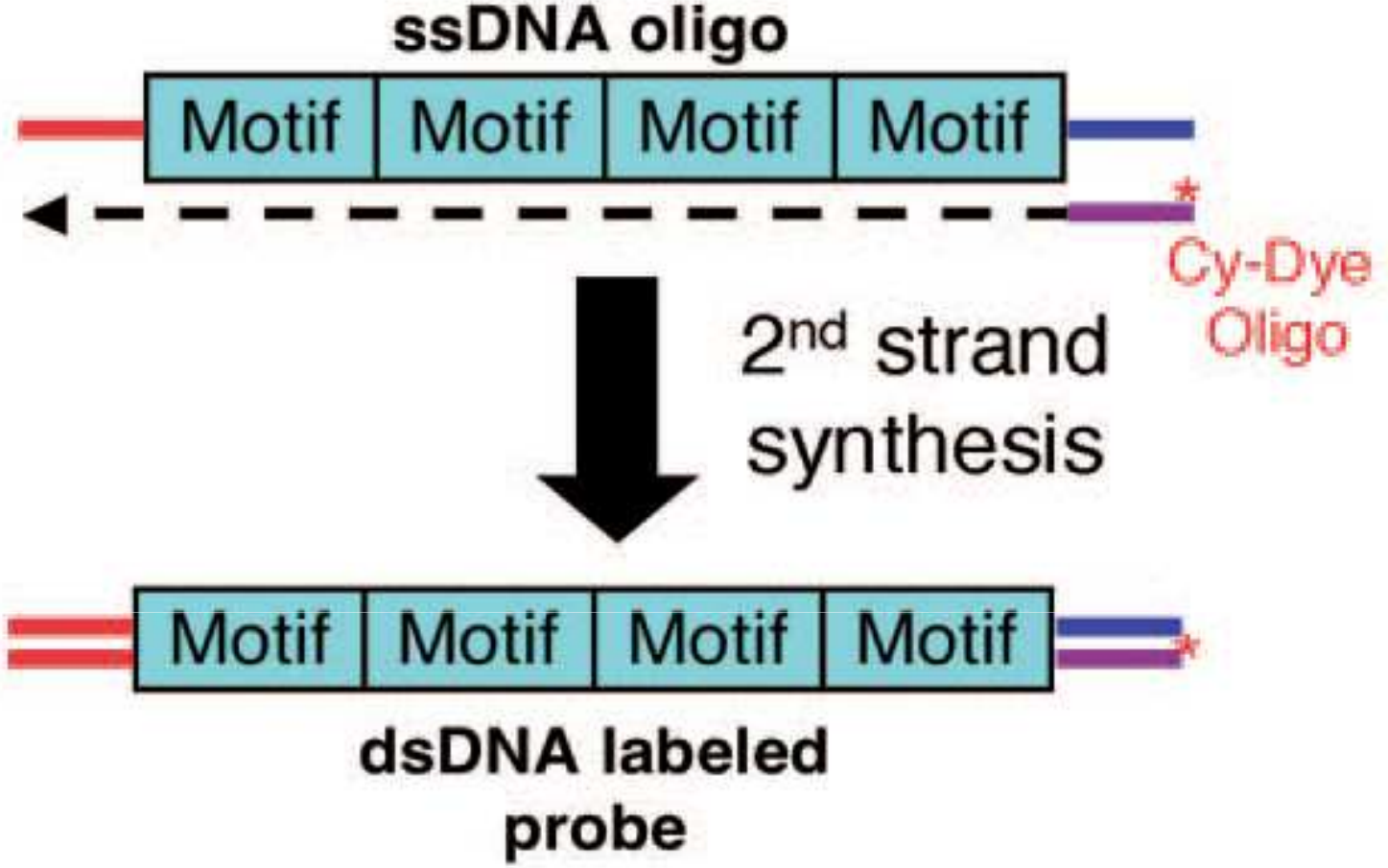

dominio
di attivazione
dominio
di legame
al DNA
DNA
sito di legame al DNA


Ser
Arg
Asn
Arg
1
2
3

(*C*) Yeast transcription factor microarrays probed with fluorescent DNA probes.

The GST-fused transcription factors purified from yeast were spotted (in quadruplicate) on each slide and probed with Cy5-labeled anti-GST



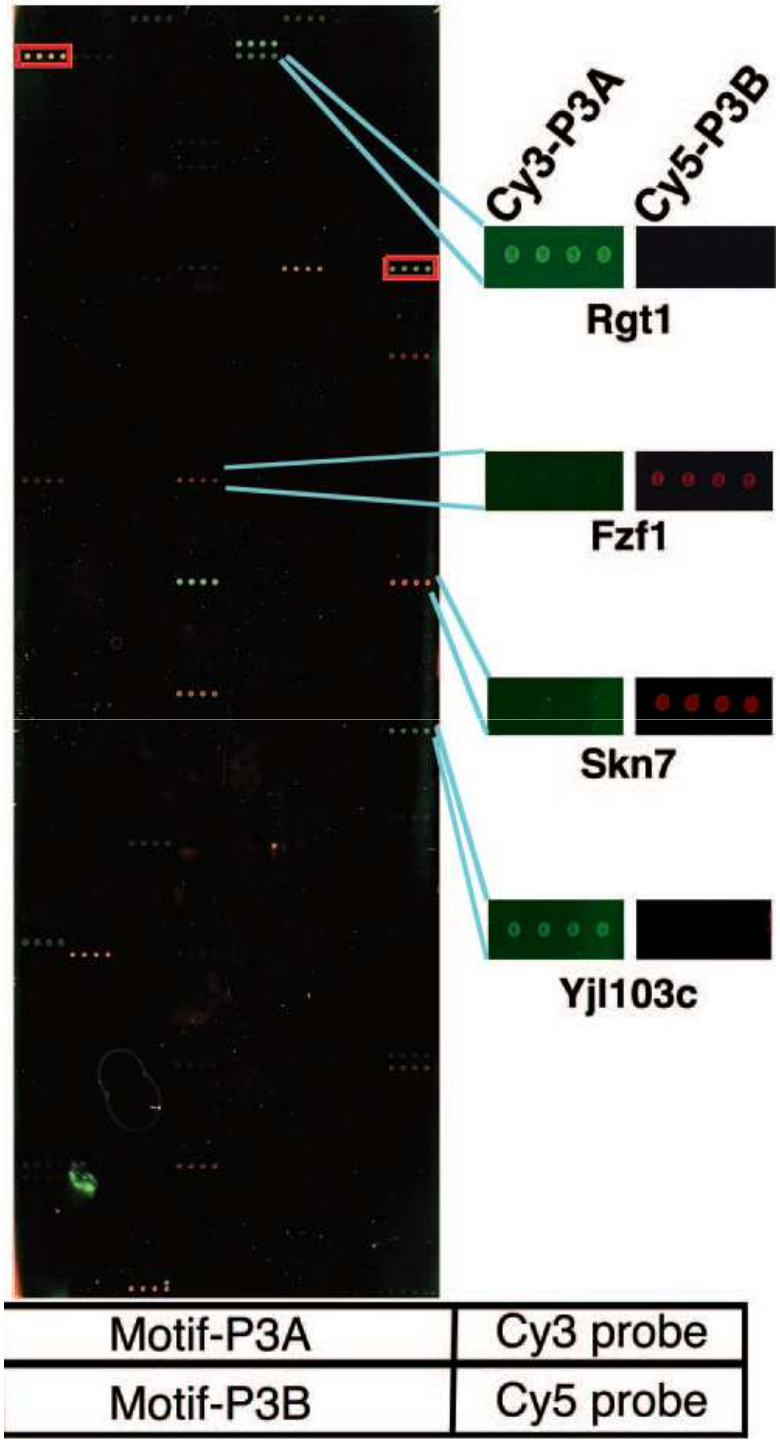


Probing the transcription factor microarray. (*A*) Probes were made by extending a universal primer labeled at its 5 end with a fluorophore on an oligonucleotide template containing conserved sequence motifs. Because the length of the sequence motifs varies and we kept the length of the oligonucleotide probes constant, three or four copies of a motif are present in each probe.

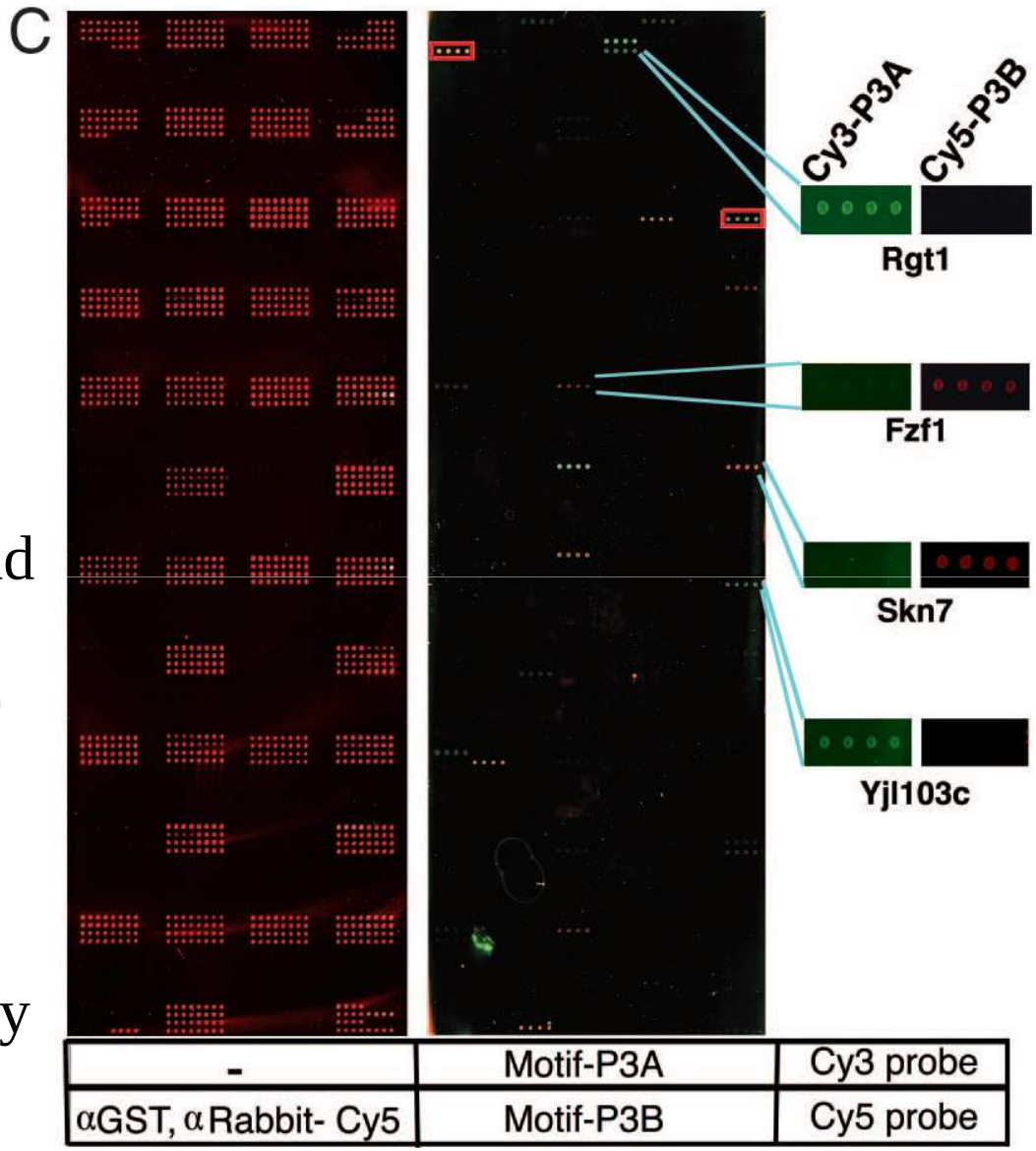
(*C*) Yeast transcription factor microarrays probed with fluorescentDNA probes.

probed with specific
Motifs (P3A or P3B)

Examples of specific DNA binding are enlarged
Es Yjl103c binds specifically to P3A but not P3B.

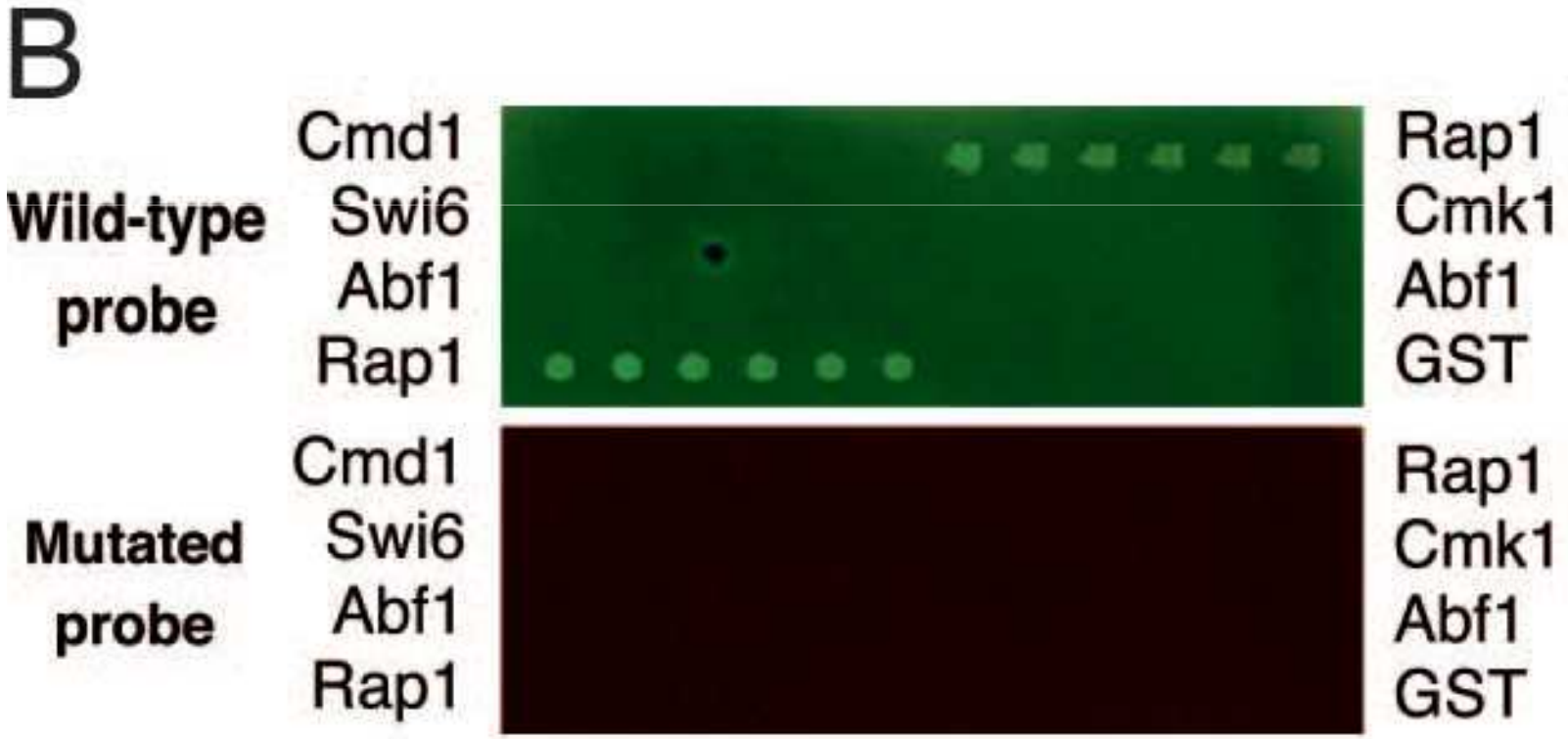


(*C*) Yeast transcription factor microarrays probed with fluorescentDNA probes. The GST-fused transcription factors purified from yeast were spotted (in quadruplicate) on each slide and probed with Cy5-labeled anti-GST (*Left*) or a pair of probes (*Right*). Examples of specific DNA binding are enlarged at the right. Yjl103c binds specifically to P3A but not P3B.



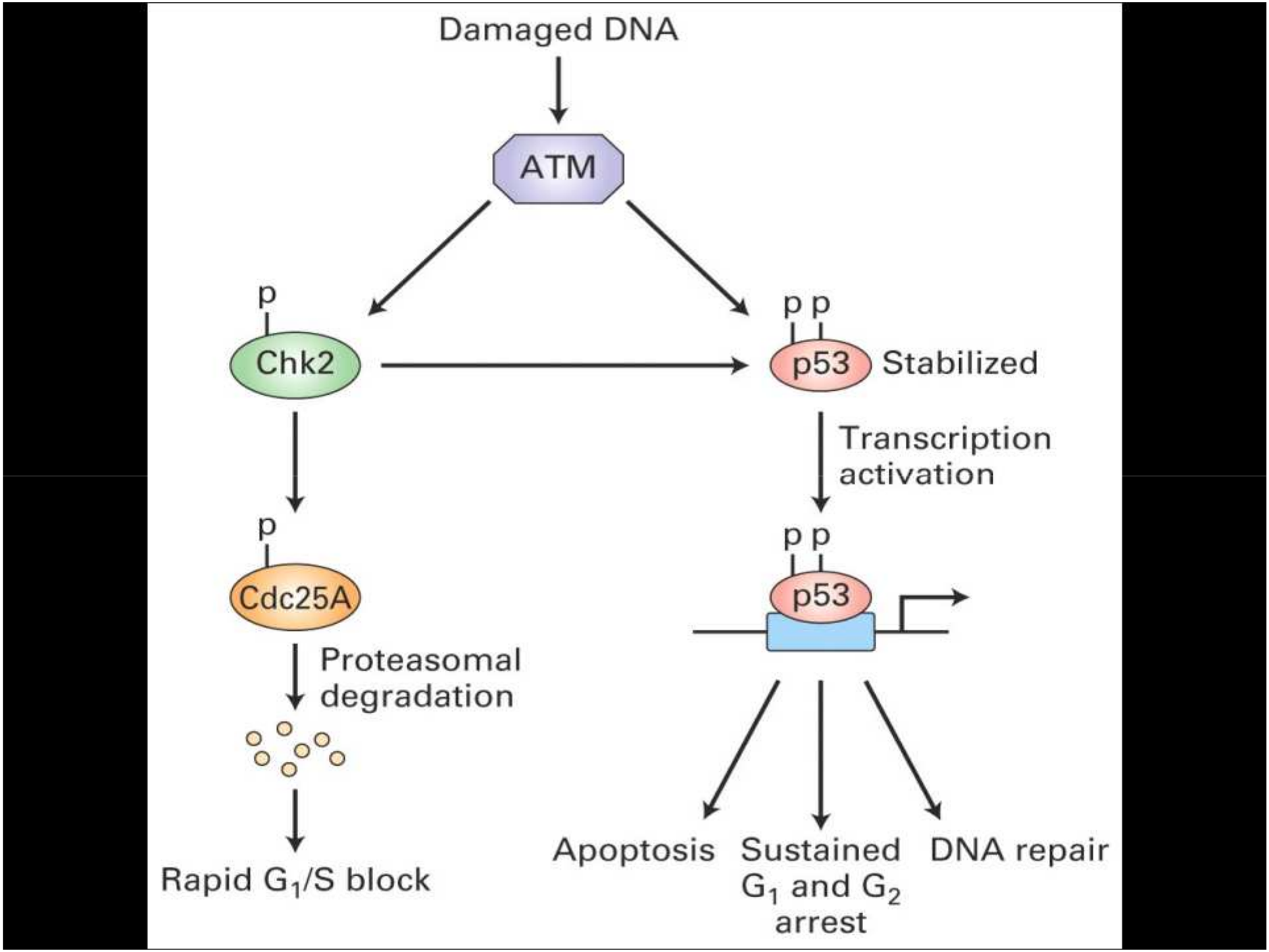
Probing the transcription factor microarray.

(*B*) Rap1 protein- a general regulatory factor that contribute to replication, silencing and telomere structure -bind to a probe containing Rap1-binding sites. Each protein was spotted six times on the nitrocellulose surface and probed with an oligonucleotide containing three Rap1-binding sites (ACACCCATGCA) (labeled with Cy3, shown in green) and a probe containing three Rap1-binding sites with two nucleotide changes (ACACttATGCA) (labeled with Cy5, shown in red).

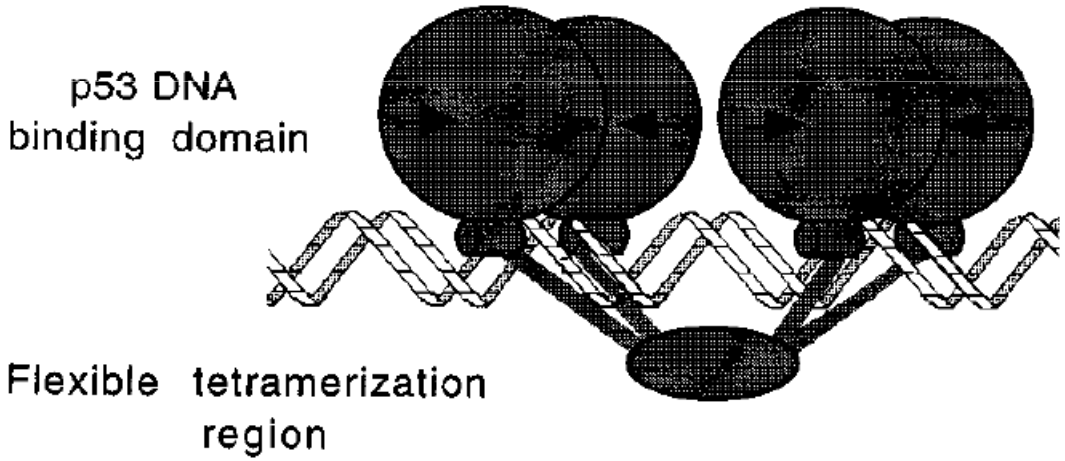


- **Protein microarrays offer a high-throughput method for determining DNA–protein interactions**

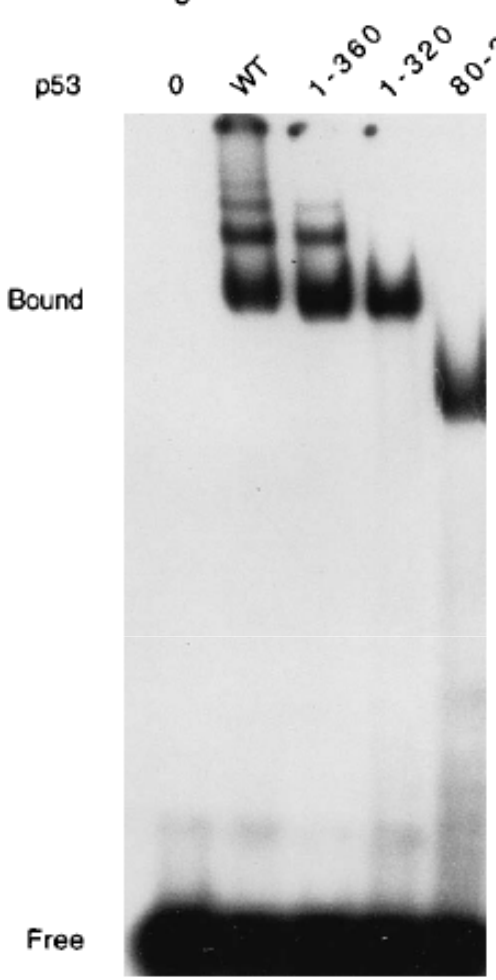
Arrays with mutant proteins


Damaged DNA
ATM
p
Chk2
p p
p53
Stabilized
Transcription activation
p
Cdc25A
p p
p53
Proteasomal degradation
Rapid G_1/S block
Apoptosis
Sustained G_1 and G_2 arrest
DNA repair

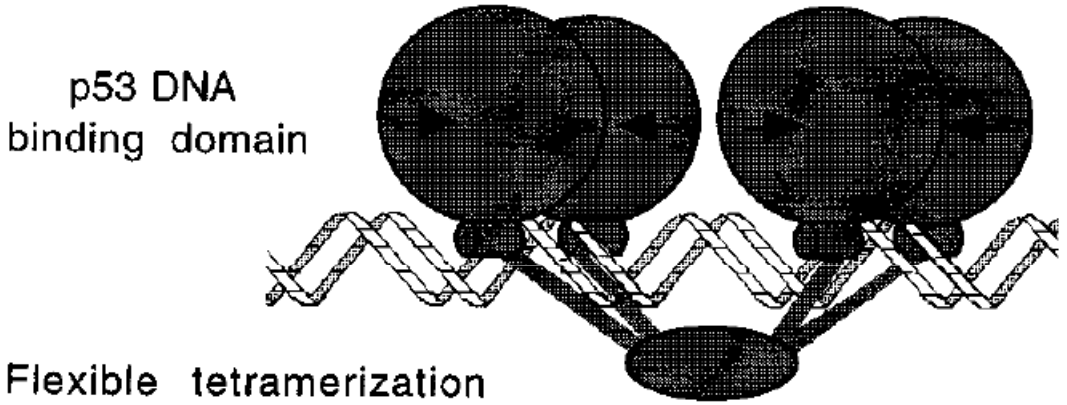
p53 BINDING TO DNA

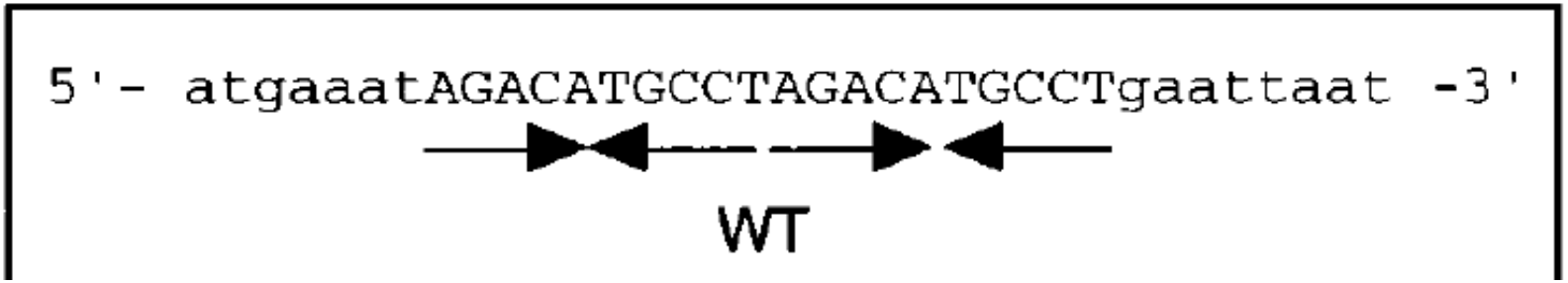


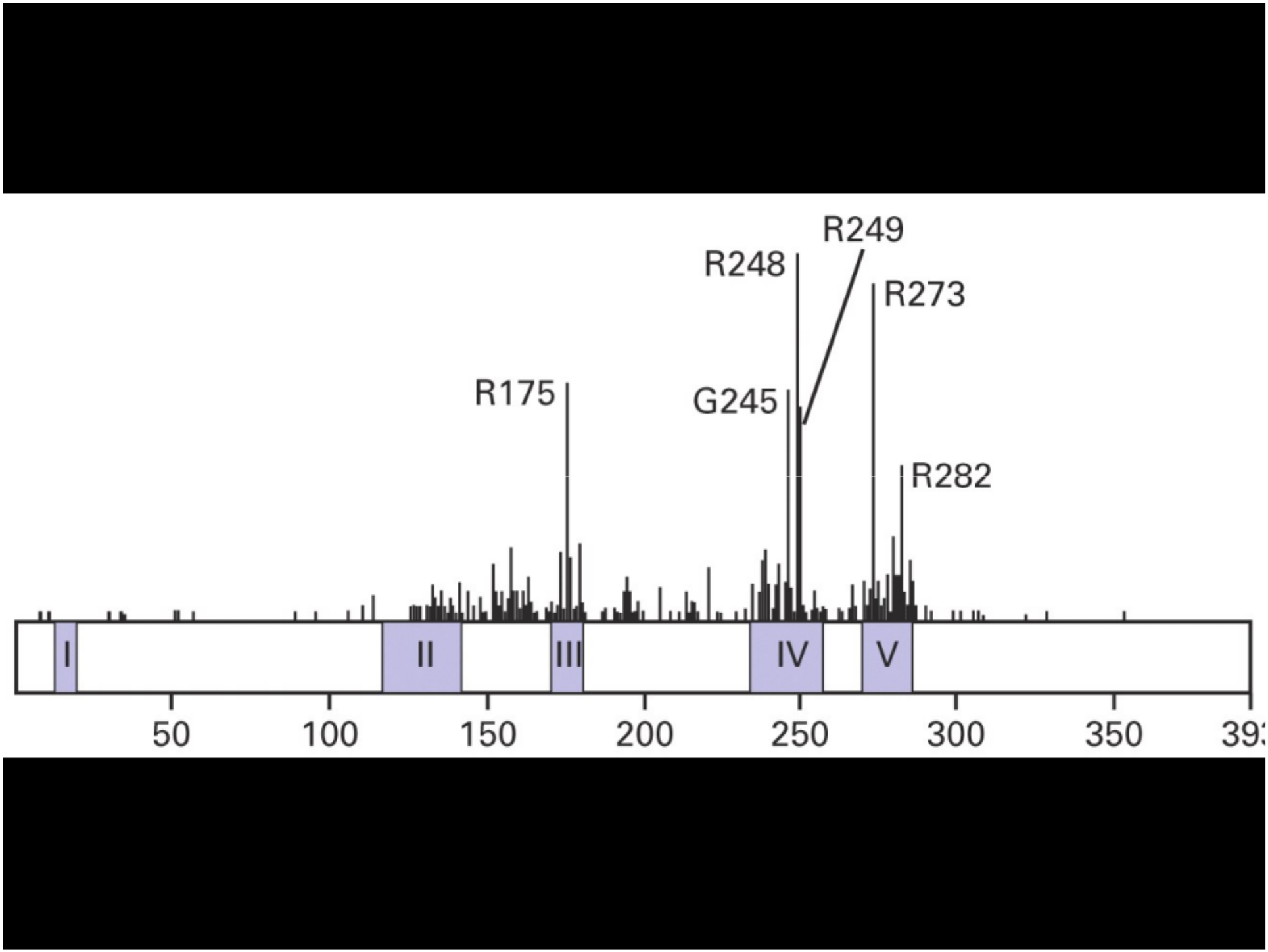
B. DNA binding



p53 BINDING TO DNA

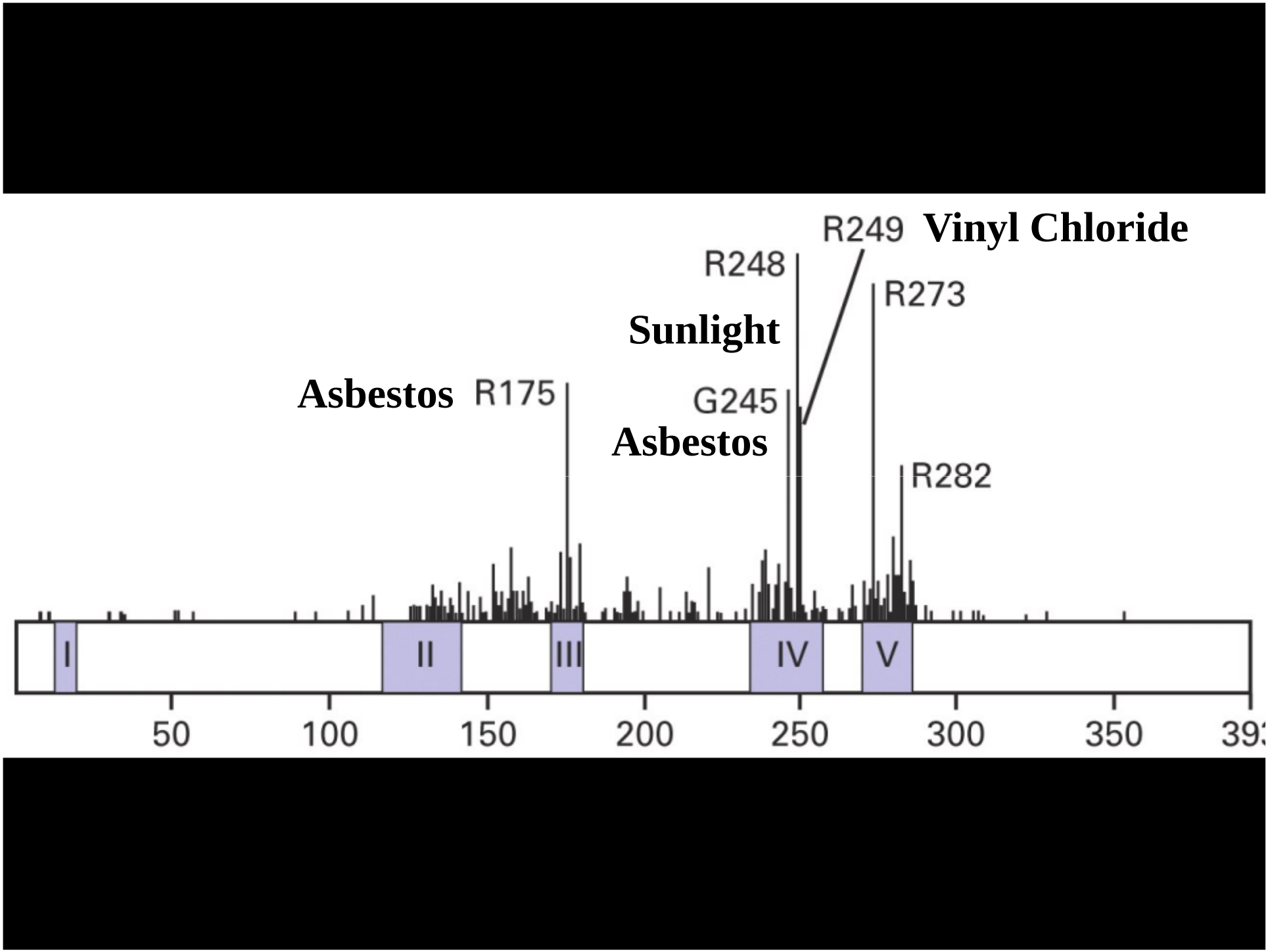




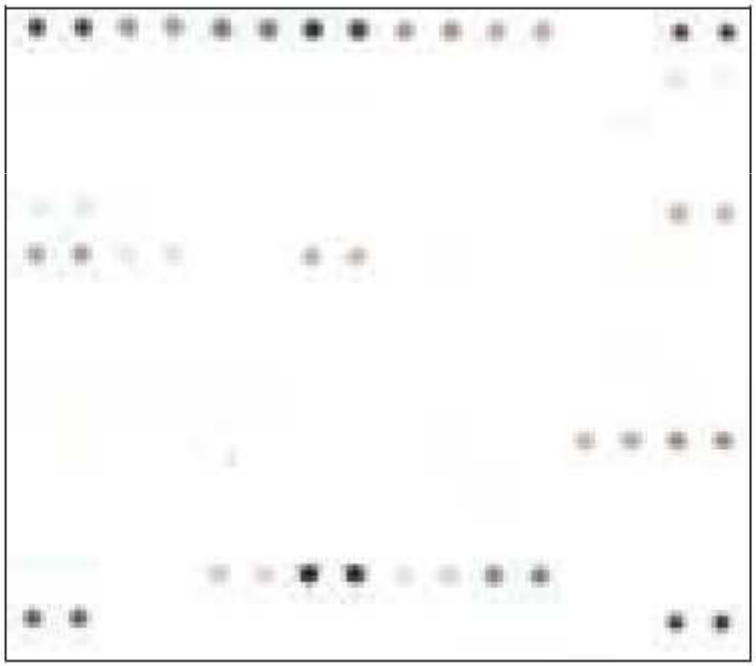

R249
R248
R273
R175
G245
R282
I
II
III
IV
V
50
100
150
200
250
300
350

Nearly half of all cancers are caused by mutations to p53 and most of these have been mapped to DNA binding core of p53 (p53C).

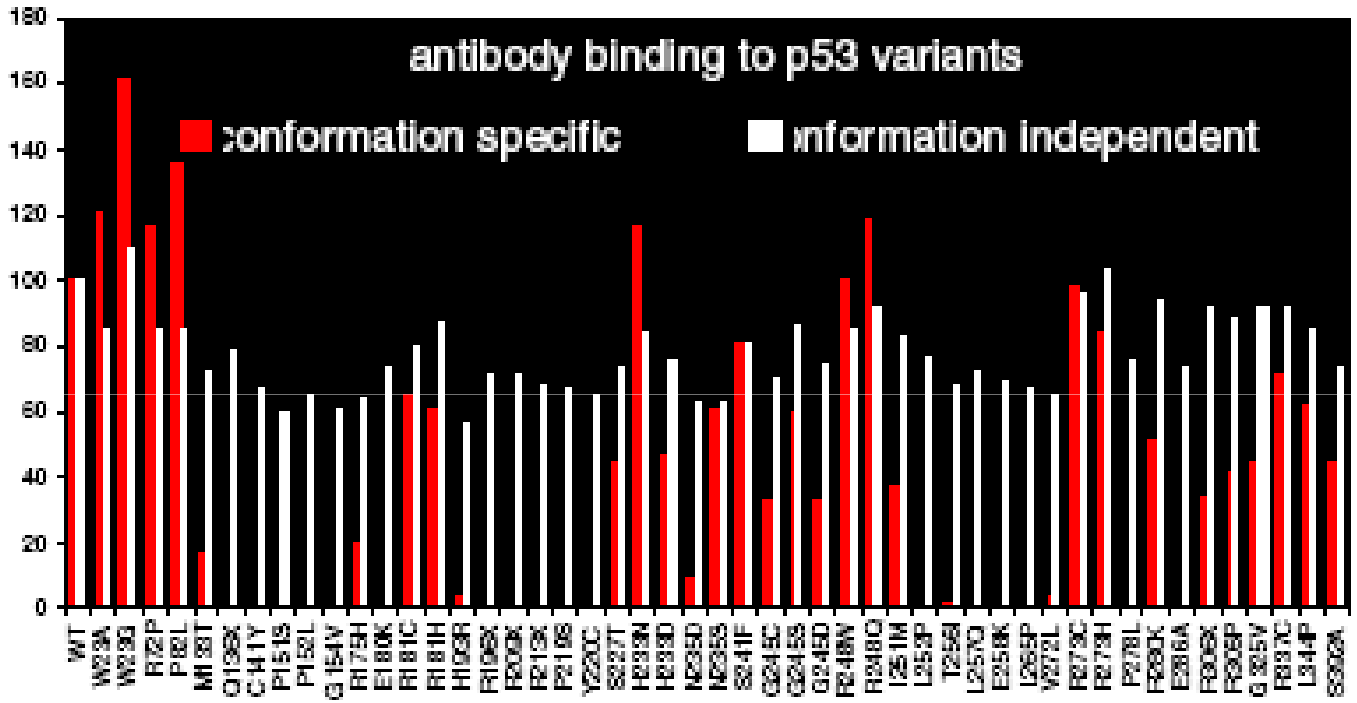
- Residues that have been observed to be the most frequently mutated areR175, G245, R248, R249, R273 and R282
- Mutations have demonstrated a decrease in the thermal stability of the structure of p53 and more importantly p53C which denatures around body temperature
- Environmental carcinogen exposure has been linked to the following mutations of p53C
- **Vinyl Chloride**
- H179L, R249W, and I255F
- **Asbestos**
- R175H, D245P, and P278S
- **Sunlight**
- G245D, R248W, and G286K


R249
Vinyl Chloride
R248
R273
Sunlight
Asbestos
R175
G245
Asbestos
R282
I
II
III
IV
V
50
100
150
200
250
300
350

Mutant p53 array

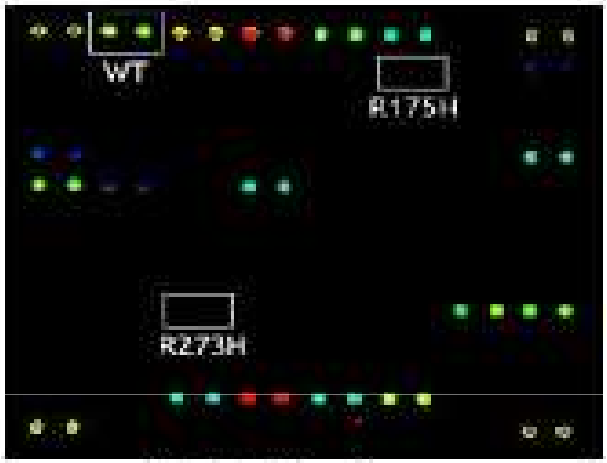


antibody binding to arrays

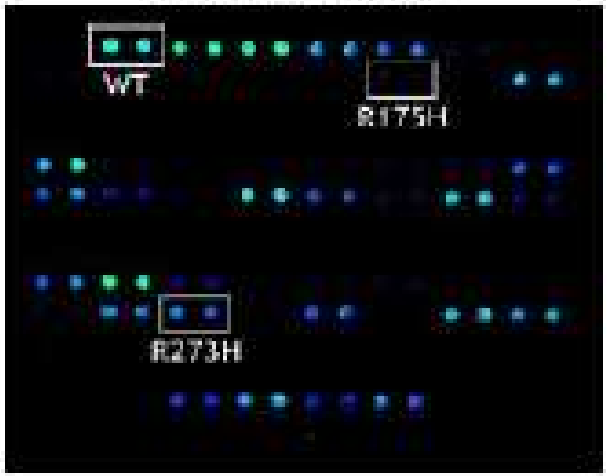


Some mutations in p53 affected the conformation of the core domain as measured by loss of binding of the conformation-specific antibody (red). However all variants were able to bind the conformation-independent antibody (white).

DNA and antibody binding compared



DNA binding



antibody binding

As expected, some mutations (e.g. R175H) affected the structure of the p53 core-domain and also abolished DNA binding. However, other mutations (e.g. R273H) showed loss of function but maintained structure. This loss of function without loss of structure indicates that these mutations changed amino acids that participate in direct contact with DNA. This is the first time such conclusions have been drawn from array technology.

The p53 network

- □□ p53 is a tumor suppressor gene. Its protein (transc. activ.) acts by :
- - Inhibition of progress through the cell cycle
- - Apoptosis
- - Inhibition of blood-vessel formation (angiogenic phase)
- - Modulating the balance between respiration and glycolysis
- □□ Is activated by :
- - DNA damage (ATM, Chk2)
- - Aberrant growth signals (p14ARF)
- - Cell stress (ATR, Casein II, …)
- □□ Is “off” in normal circumstances. Produced at some rate but degraded by ubiquitin labelling (MDM2, ...)
- □□ Is activated through inhibition of degradation
- □□ It activates its own controller
- □□ In many tumors (~ 50%) it is found to be mutated
- □□ In a number of cases normal p53 cannot achieve control
- □□ A very complex network of interactions (an highly connected module in the mammalian cell cycle control and DNA repair system)

GADD45

- Growth Arrest and DNA Damage 45-alpha (GADD45-alpha) is a nuclear protein involved in maintenance of genomic stability, DNA repair, and suppression of cell growth through interaction with nuclear elements, including cyclin-dependent kinase inhibitor 1A (CDKN1A) and PCNA.

Figure 1. An example of a typical array image for the Cy3-GADD45 DNA binding assay is shown below (see Appendix B for array layout).

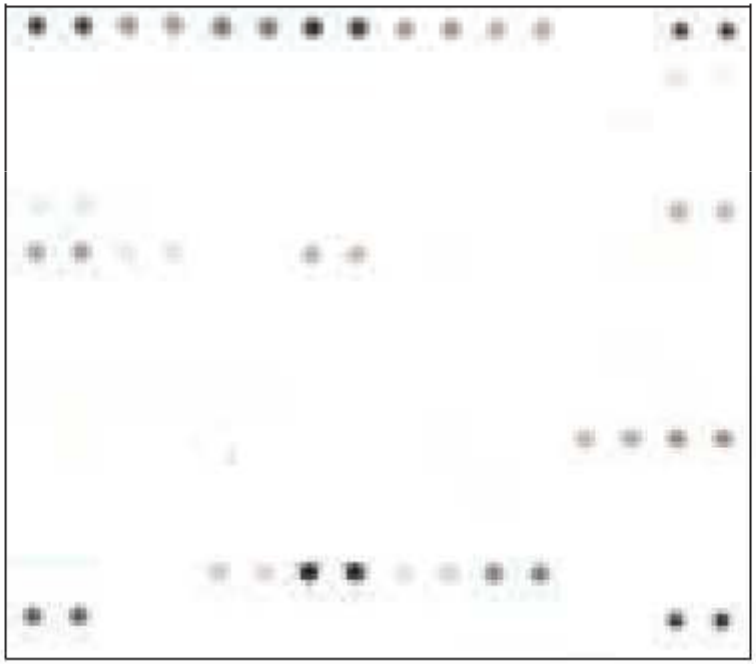


Figure 2. An example of a typical profile for the Cy3-GADD45 DNA binding assay is shown. The data was obtained by normalizing the binding exhibited by mutant and control proteins to the wild-type p53 binding.