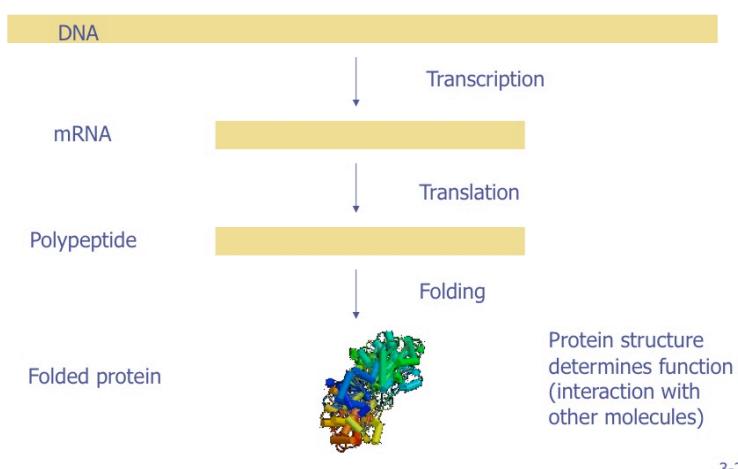


Part 3

Protein Expression and Function

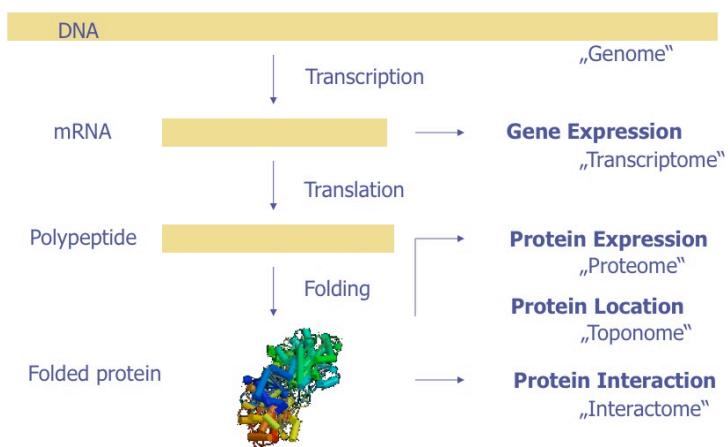
1

Gene to proteins



3-2

Expression analysis



3-3

Gene expression and cell specialization

(1) Expression is different in different cell types

- Same genome
- Each cell type produces the proteins it needs
- Expression differences reveal function of the different gene products

(2) Expression differences may indicate pathology

- E.g., cancer cells show expression changes to normal cells
- Is of diagnostic value (might discover cancer at the cell level)
- Shows functional mechanisms of cancer (which proteins are responsible for the new behaviour)

3-4

Expression and dynamics

(1) Expression is a dynamic process

- Expression levels vary over time
- Internal regulation, e.g., in cell cycle control
- External regulation in response to signals or changing conditions

(2) Expression regulation is robust

- Cell function can tolerate fluctuations in expression levels
- Thus even large differences in expression do not necessarily imply different function

(3) Expression takes time

- Protein expression may take minutes or hours
- Isolated snapshots might be misleading

3-5

Expression snapshots

(1) Expression measurement must be „quick“

- To get an accurate expression profile at time t, cell processes have to be interrupted
- Otherwise, chemical reactions will continue in an uncontrolled manner before the actual reading can be taken

(2) Sample preparation is crucial

- Usually, many cells are taken together and are destroyed, e.g., by centrifugation
- Processes at a time level of seconds and even minutes might still happen (e.g., stress response to centrifugation)

(3) Multiple snapshots are needed

- Over time
- Over different external conditions

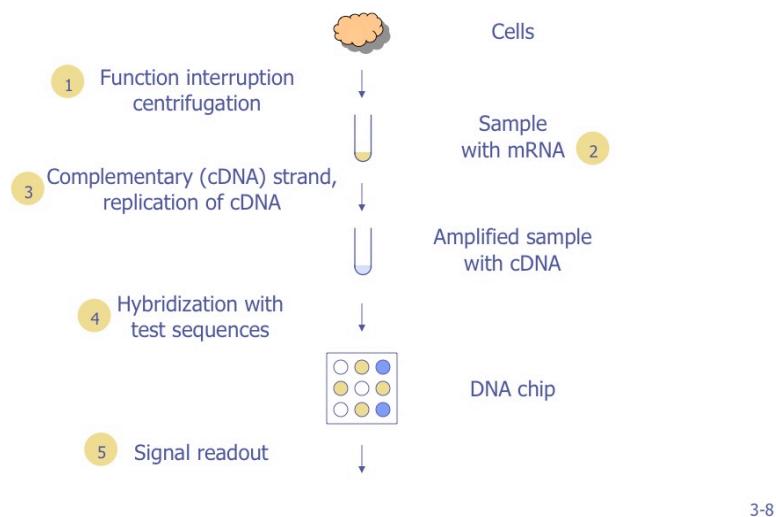
3-6

3.1

Gene expression analysis

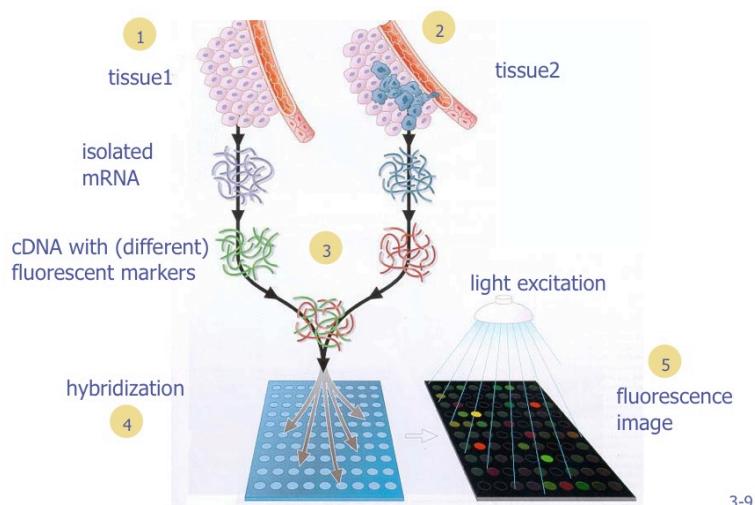
7

Gene expression experiments



3-8

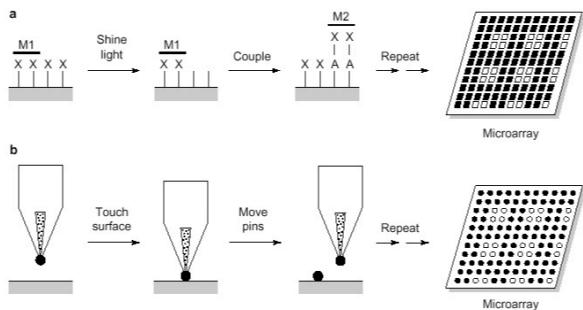
Hybridization experiment



3-9

Microarray technologies

Photolithography (X = photocoating, stepwise by base)



Liquid spotting (of complete DNA molecules)

3-10

Photolithography

(1) On-chip synthesis of DNA

- An optical mask selectively removes light sensitive coating
- Free places are flooded with one base
- Enzyme couples it to previous bases
- Need 4 masks (4 bases) per element: expensive and time-consuming!
- Can be directly generated from a database

(2) Affymetrix chips

- Short oligonucleotides (25 base pairs) per spot
- Because of long production time
- Longer sequences are matched through multiple oligonucleotides
- Software needed for correct oligo design, so that each gene has about 16 or more specific matching oligos

(3) Alternative: Ink-jet printing

- Piezoelectric (focused) spraying of individual bases

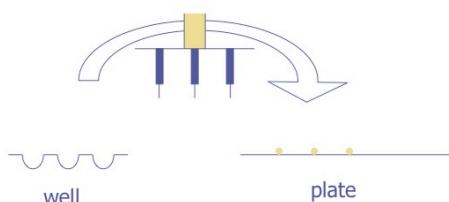
3-11

Liquid spotting

(1) Robotic pipetting from a library

- On glass substrate or nylon filters
- Each sequence needs to be synthesized
- Problem of contamination
- Problem of correct synthesis

(2) Equipment is already present in most labs



3-12

Hybridization

(1) Need to get rid of ribosomal RNA

- Can be 80% of total cell RNA

(2) cDNA is tagged with fluorescent dye

- Two samples can be mixed with different colours
- Radioactive labeling also possible

(3) Sample cDNA attaches to probe sequence

- Hybridization errors: may also attach to similar sequences

(4) Two probes for each sequence

- Perfect match (PM): identical sequence
- Mismatch (MM): one different base in the middle
- Actual signal is difference between perfect match and mismatch (sort of background noise from a number of similar sequences)

3-13

Readout

(1) Laser excitation

- Focus sequentially on individual pixels to avoid background noise from scattered light
- Optical detector readout
- Many pixels per spot = slow

(2) Light excitation

- Simultaneous lighting of whole chip
- High resolution camera
- Fast, but lower signal-to-noise ratio
- Possible for lower density arrays

(3) Phosphor imager

- For radioactive readout

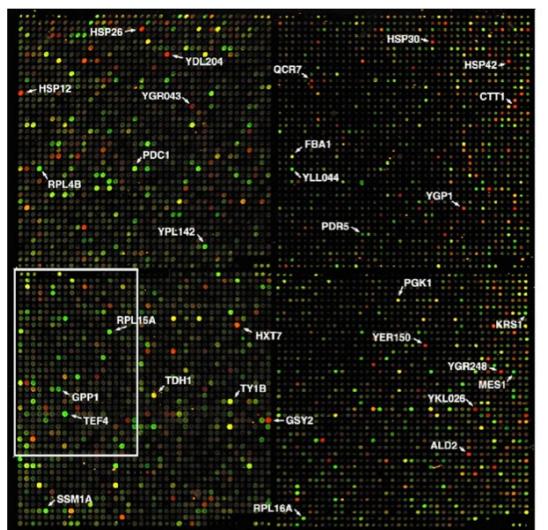
3-14

Example

6400 yeast DNA sequences
printed on a glass slide 18 x 18 mm

Two samples
were prepared
with red and
green dyes,
respectively

From: deRisi et al,
Science 278, 1997,
680-6.



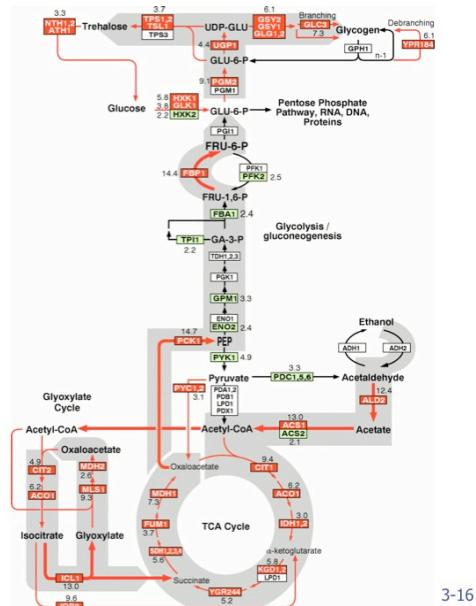
3-15

Diauxic shift

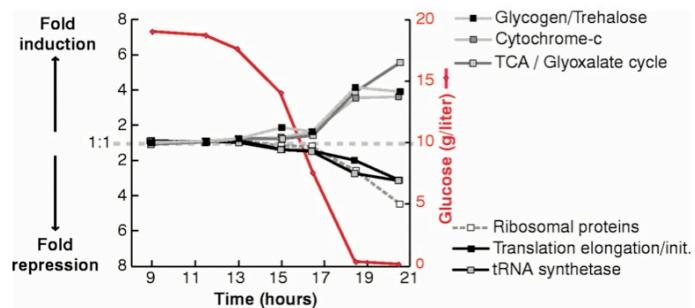
Yeast shifts from producing ethanol to consuming ethanol when glucose is depleted

Red: Increased expression

Green: Decreased expression



Results from the expression experiment



From: deRisi et al, Science 278, 1997, 680-6.

3-17

3.2

Proteomics

18

Proteins after Translation

(1) Degradation

- To create equilibrium between synthesis and degradation

(2) Posttranslational modifications

- Individual amino acids are modified (e.g., phosphorylation)
- These modifications may activate/deactivate function

(3) Transport

- Into different parts of the cell or outside the cell

(4) Assembly of protein complexes

- Often required for protein function

3-19

Proteomics

(1) Quantitative analysis (expression proteomics)

- Gel electrophoresis (old)
- Quantitative mass spectrometry of protein mixtures (new)

(2) Posttranslational modifications

- Mass spectrometry to detect weight differences

(3) Localization (topological proteomics)

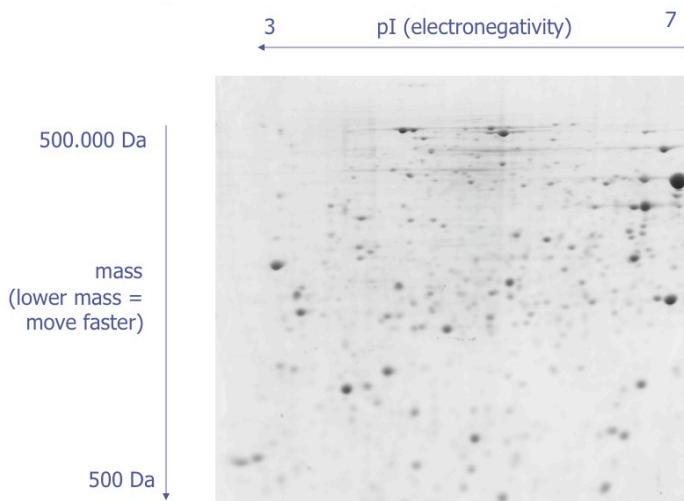
- Selective purification of organelles
- Microscopy with fluorescent markers

(4) Complex assembly (interaction proteomics)

- Affinity purification of complexes through baits
- Bilateral interaction (yeast two hybrid screen, protein chips)

3-20

Gel electrophoresis



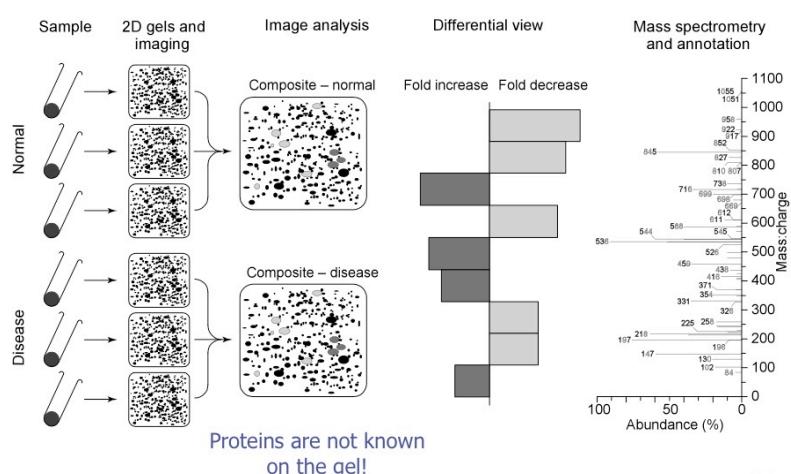
3-21

Electrophoresis preparation

- (1) Sample preparation
- (2) Electrostatic separation on strip (pI)
- (3) Mass migration in gel chamber (vertical)
- (4) Staining (silver or fluorochromes)
- (5) Scanning (flatbed or camera)

3-22

Electrophoresis experimental approach



3-23

Problems in Gel Electrophoresis

(1) Reproducibility

- Spot intensities and locations vary even for identical gels created in the same processing runs.
- Not all proteins easily make it onto the gel, and different proteins stain in different ways, possibly in a non-linear relation to concentration. Thus, concentrations of different proteins are not easily comparable.
- Not all proteins are chemically stabilized so that ongoing reactions cause a smearing of certain proteins over a whole area.

(2) BUT

(3) Electrophoresis is the only technique that allows quantification of a large number of proteins at the same time

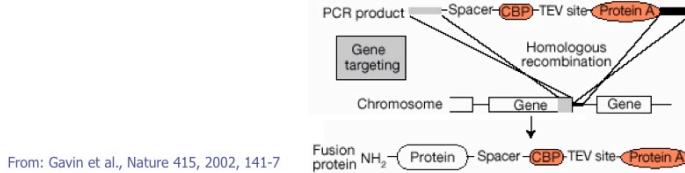
Affinity purification

(1) Reduce the number of proteins and image purity

- Through selecting only a subset of proteins from the sample
- E.g., selective centrifugation
- E.g., membrane only

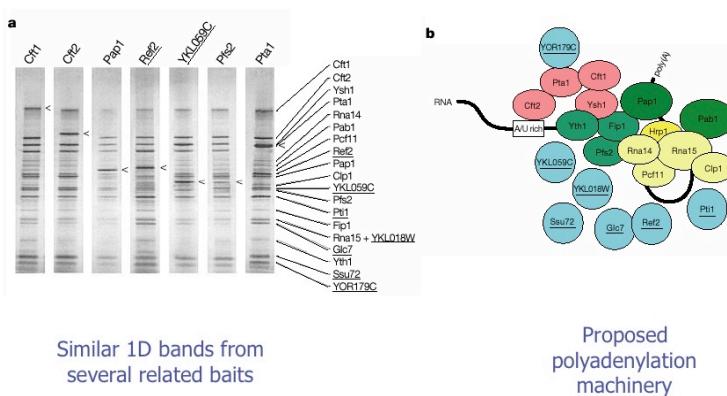
(2) Bait

- (Genetically) tagging particular proteins (e.g. in yeast)
- Selecting from the sample (gently) all proteins that couple to this protein (partial complexes)



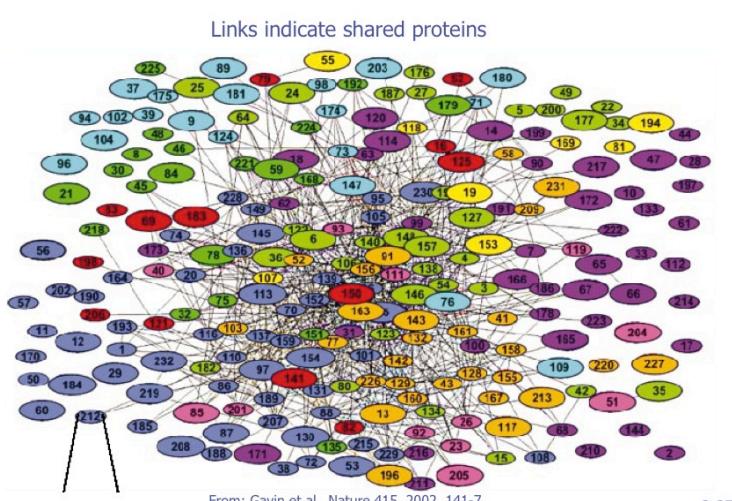
3-25

Polyadenylation machinery in yeast



3-26

Protein complexes in yeast



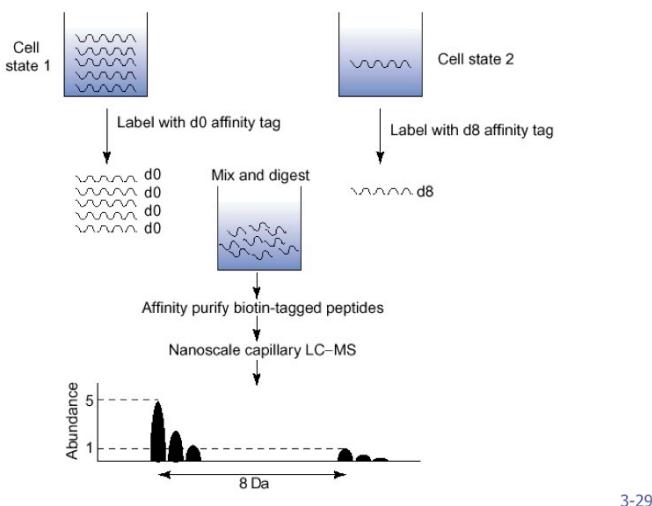
3-27

Protein mixtures by MS

- (1) Each protein in the mixture is identified by a single tagged peptide
 - MS/MS identifies peptide sequence and thus the identity of the parent protein
 - Requires constrained protein mixture
- (2) Mixture is further separated by liquid chromatography (LC)
 - Mass-based separation of peptides with direct input into MS
- (3) Relative abundance by peak difference between two different tags
 - Different weight tags for test and reference sample

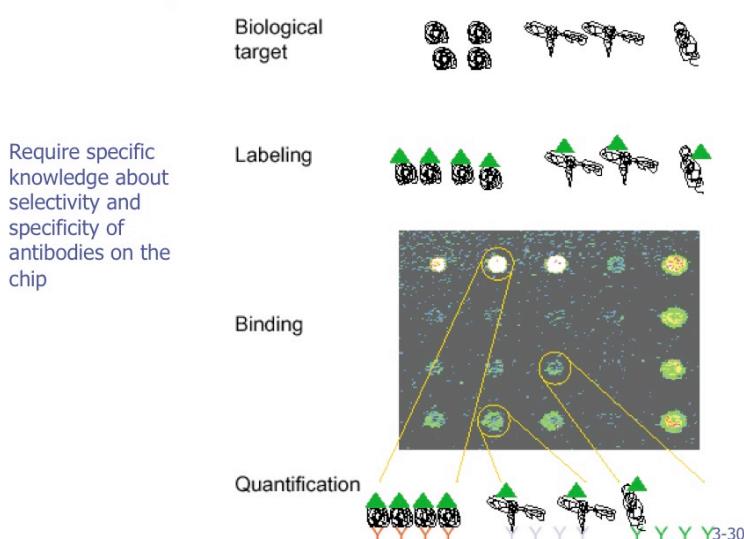
3-28

Isotope-coded affinity tagging (ICAT)

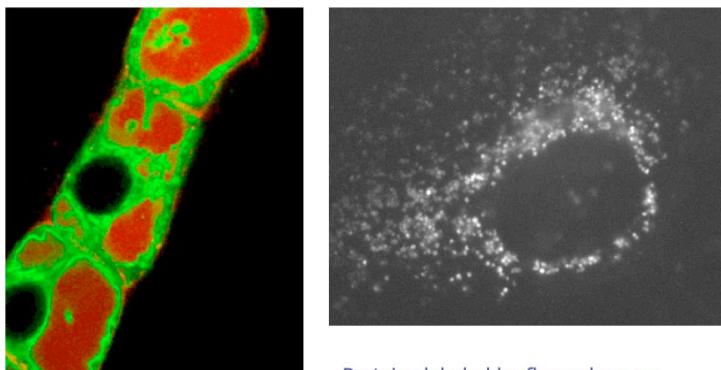


3-29

Protein chips



Protein localization by microscopy



3-31

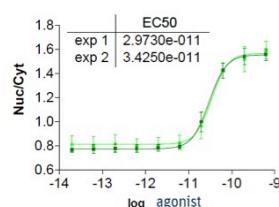
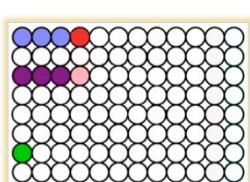
Toponomics

- Topological Proteomics
 - Measuring spatial distribution of some/all proteins
 - Also called topoproteomics/location proteomics
- Toponomics
 - Toponome gives the laws of spatial arrangement¹
(not necessarily causal)
 - Modeling spatial distribution based on measurements
 - Reducing observed spatial distribution into representative descriptions

3-32

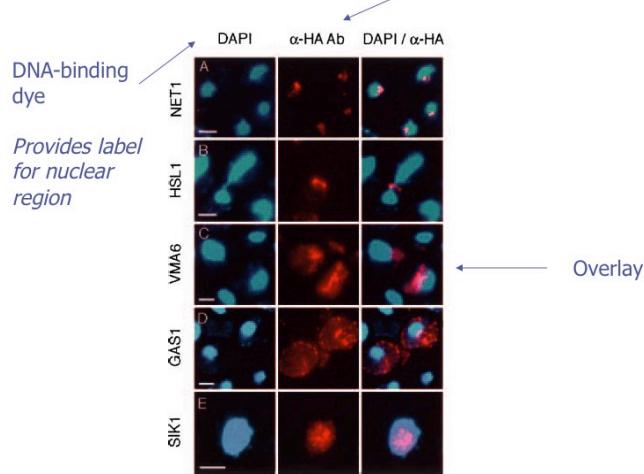
High Content Screening

- Automated microscopy of cellular events
 - Input: System perturbation
 - Output: Population response
- Screening = Optimization of input
 - Using toponomics model to derive numerical descriptor
 - E.g., dose-response curve
 - Black-box model with respect to molecular mechanism



3-33

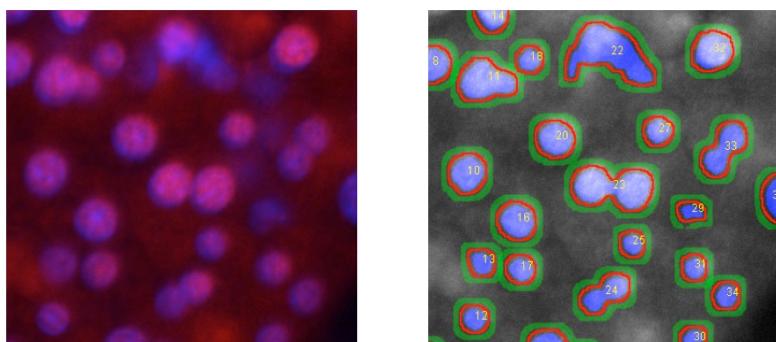
Subcellular localization



From: Kumar et al., GENES & DEVELOPMENT 16(2002):707 –719

3-34

Nuclear translocation



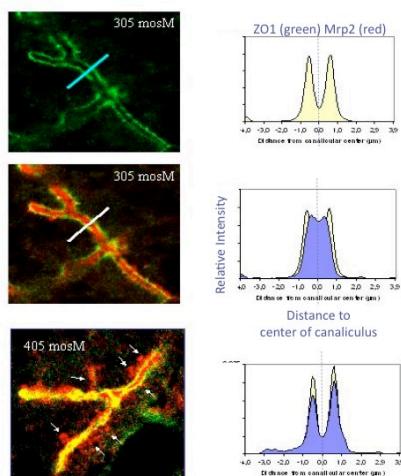
1. Nuclear region segmentation (adaptive threshold)
2. Region extension
3. Quantification

3-35

Toponomics of transport

Dual labels

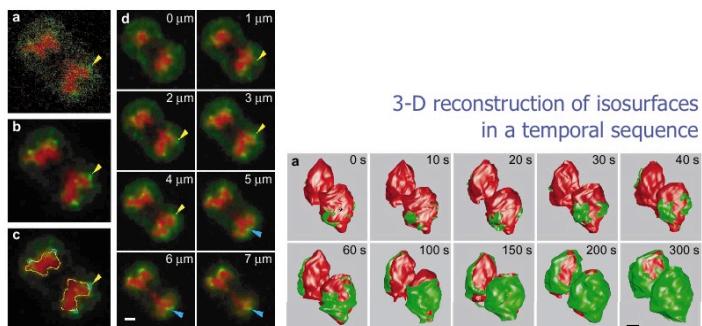
- Topological markers: ZO1 is a tight junction protein, indicates structure
- Functional markers: Transport protein is regulated through translocation
- Spatial distribution (profiling functional marker orthogonal to structure) gives information about regulation state



3-36

Dynamic 3-D imaging

Image slices with two different fluorochromes (red and green)



From: Gerlich et al., Nature Cell Biology 3(2001):852-855

3-37