Some Approaches to the Study of Sub-cellular Biology

Autoradiography

Fig. 8.3

- Early studies
- Incubation with radioactive amino acids
- Pulse-chase, various times
- Fix, expose film
- Metabolic fate of protein visualized on autoradiograph.

Endomembrane Concept

Fig. 8.3

- Studies show membrane structures as interrelated in a dynamic system
- Leader sequences: Amino acid 'zip codes' provide 'addresses'
- Materials are transported via transport vesicles
- Biosynthetic pathway modifies proteins
- Secretion: constitutive, regulated, membrane recycling.

GFP-green fluorescent protein

- gene isolated from luminescent jellyfish
- gene product absorbs blue light/emits green light
- GFP gene inserted into expression vector will transform cells, fluoresce.
- can be tagged onto another gene
- can follow fate of gene product thru cell cycle by periodic exposure to blue light.--non destructive
- new types of GFP emit at different wave lengths--what is advantage of this?

Recent Experiments with GFP. Re: Biochem. J. (2001)357, 529-536

- No GFP tag no PM label
- positive fluorescence on PM with GFP beta tag
- positive fluorescence on PM with GFP alpha tag
- antibody stain is positive
- GFP advantage: non-destructive, dynamic

(understand terms in title and results given here)

Cell fractionation by differential and density gradient centrifugation: Enrichment for a phloem-specific protein.

Methods:

- disrupt tissue

- differential centrifugation
- PAGE fractions for enrichment
- load enriched fraction on density gradient
- dialyze fractions
- PAGE
- electron microscopy of enriched fractions

Sub-cellular localization of a phloem-specific *lectin*. A: lanes 2-5 pine phloem; lanes 6-9 pine xylem. Top, *SDS-PAGE*; bottom, *western blot*. B: *immunogold localization* on phloem protein crystals. C & D: *purified protein* (by *glycosamine affinity column*).* (Re: Planta (1989) 179: 506-515)

- *understand all italicized terms

Immunolabelling

- Requires protein purification or leader sequence
- Antibody/antigen interaction
- Sensitivity enhanced with fluorescent tags.
- Figure: immunofluorescence of pre-mRNA splicing factors in nuclear subcompartments during interphase

Mutants

Fig. 8.7

- Yeasts are major subjects for cell bio studies
- Single cell
- Can grow as haploids (no masking mutation)
- Mutants deficient in secretion used (*SEC* genes)