Jon Kuhn

E. coli

v. low variation in trp, lac

-attB (λ attachment site) - 250 bp region
- stop codons in all frames
- "junk" DNA

-gal KTE - attB - ORF - bioA

- all attB sites were identical
- in intergenic ~~debeds~~ region
  - all but one identical
  - the other one has six substitutions

- ORF_{BIO} - no homology

J. Freidman

Principle
- information for folding is in sequence (Anfinsen)

unfolded —FAST (driven by hydrophobics)→ collapsed —SLOW→→→ folded

Chaperones - seem to bind folded intermediates

- GroEL, HSP 60's
  - inducible by heat, chemical denaturants
  - homo-oligomer (14 subunits)

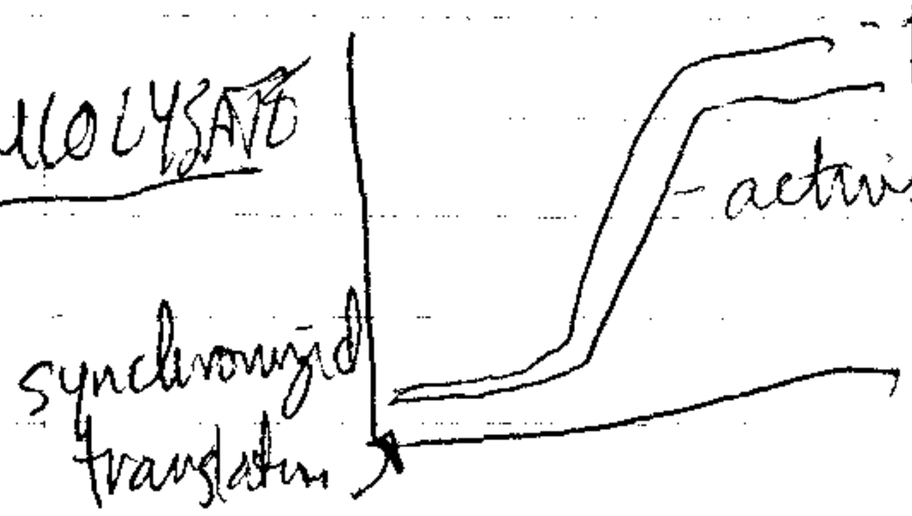
Eukaryotic Homolog?

- biochemical assay - firefly luciferase
- isolated eukaryotic version
- one protein is TCP - so called complex TRiC
- made up of ~~some~~ different components
  - peptide sequence
  - all sim. to each other but unique
  - sim. to GroEL sequences
  - most similar in nucleotide binding regions

BUT
- normally folding occurs in order during translation

∴ use in vitro translation system

RETICULOLYSATE



} small lag time which increases as T° is lowered

PROTEASE RESISTANCE - usually due to completi folding
- resistance correlated w/ activity (sm. lag)

ARE CHAPERONES INVOLVED?

non-cleavable ATP?
- stabilized partially made ribosome + protein
- to get stable product - needed ATP

Others
Hsp70 - used for translocation across membranes
Hsp40 - helps Hsp40
TriC
Hsp90

Axel F

Dosage Compensation

- Drosophila = 2X transcription ♂ as ♀ on X

are there female specific lethals

Genes

MLE
MSL1
MSL2
MSL3
} all bind to same region of X chromosome
all bind cooperatively

- Histone H4 also binds there

♀
X:A = 1

SXL ON

no dosage comp.

♂
X:A = 0.5

SXL OFF

dosage compens. on

- what genes are dosage compd ?
- what genes are not ?

- use to search for motifs

RAD8
RAD5
RAD16

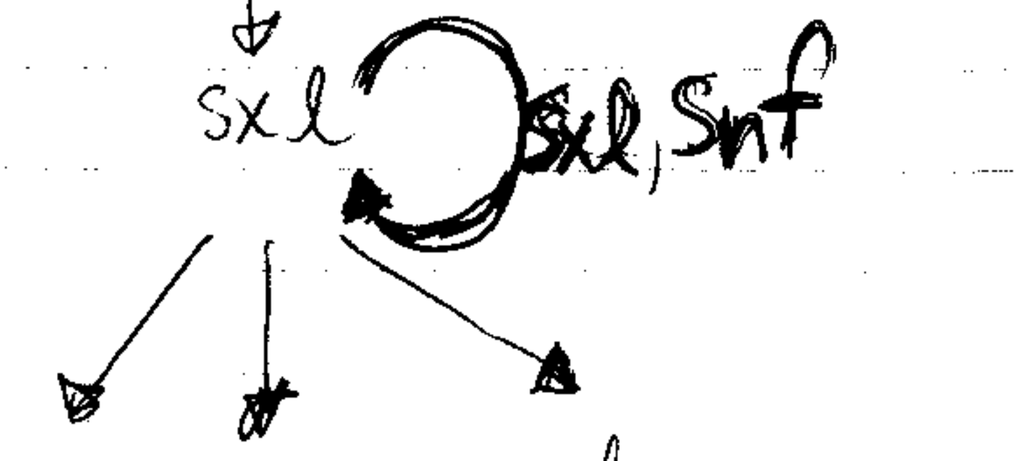
Greg

Msls lethal to ♂ (homozygous)

fx. in msls is low

X:A

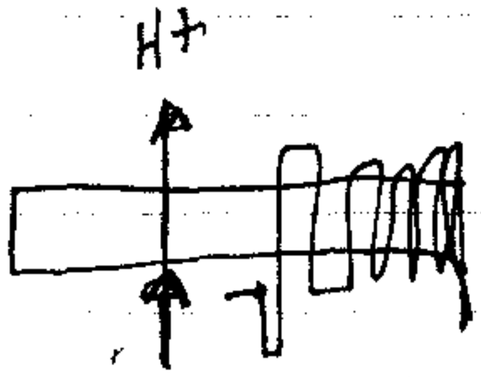
da, her, sis-a, sis-b ...



PARRAS
COLLEEN

Calcineurin

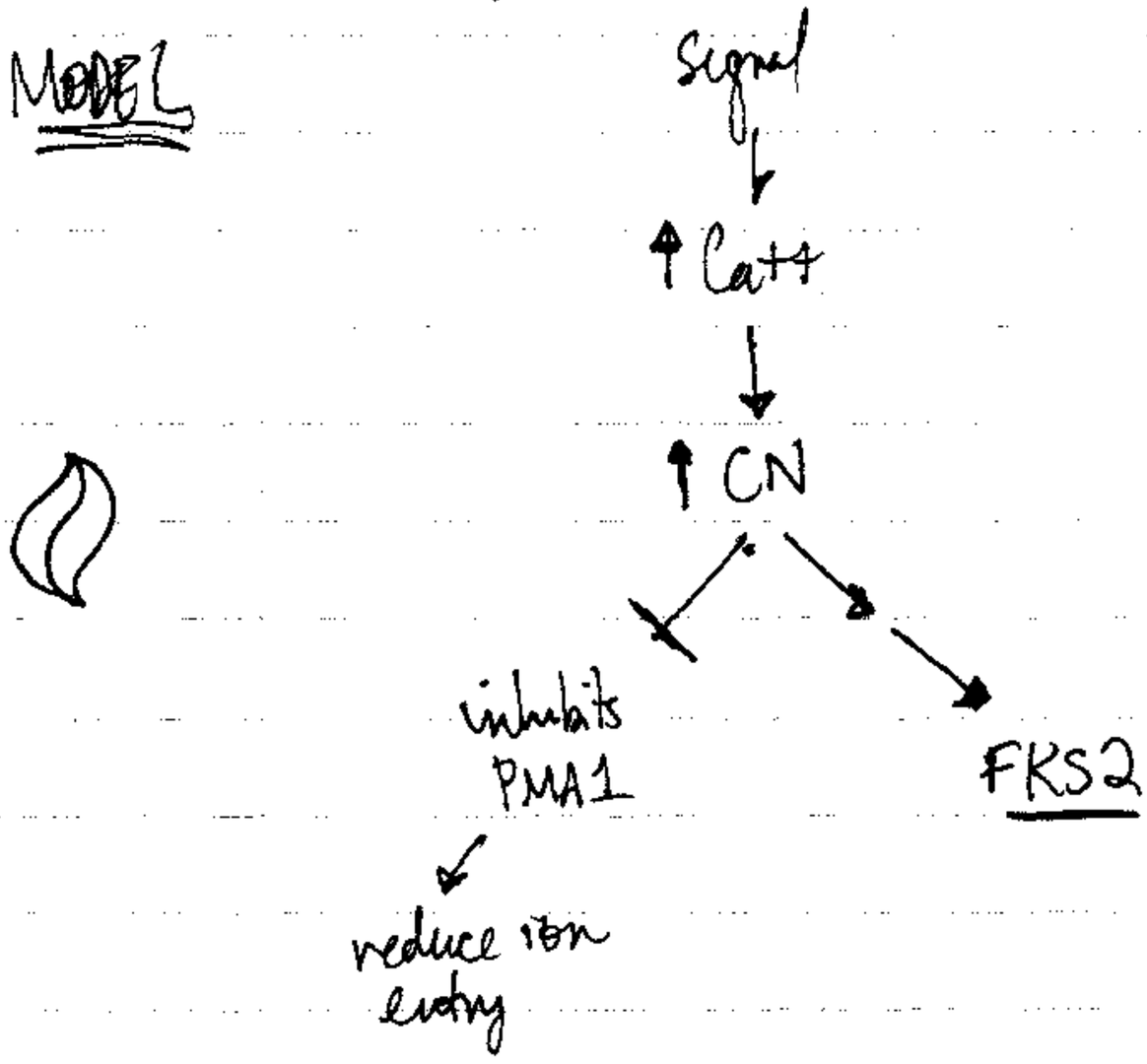
pma1
- hydrolyzes ATP
- creates proton gradient



- mutations in this gene incr. ability of Calcineurin mutants to grow on Na^+

Calcineurin
- may be a stress sensor

MODEL



HUM1
Na^+/Ca^{++} exchangers } limited seq. identity but similar membrane topology

TE1

Calcineurin = PP2B

· duct is for enzymes

Synthetic lethals
- mutations that in Calcineurin mutant background are lethal

FKS1 FKS2

① CND1 = FKS1 = ETG1 : CWH53
- required for B 1 3 glucan synthesis (in cell wall)
- another gene (FKS2) highly similar
- double mutants lethal

FKS2

② CND2 = KRE2
- another gene v. similar = SKN1
- involved in synthesis of B, 1 6 glucan (in cell wall.

- many other mutants in cell wall synthesis are calcineurin dependent mutants

Ron Kopito

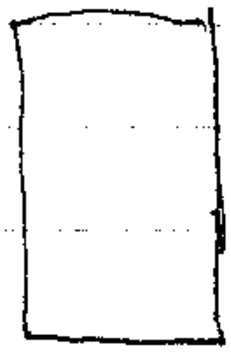
CF
-Cl^- channel problem
-channel is blocked

(in cholera Cl^- channels are constitutively on)

CFTR
=ABC transporter family
=includes
STE6 = moves ~~Baa~~ peptide
mdr
TAP = pump peptides
ALDP
HisP
HylB

by UVA

CFTR = Cl^- channel
- >400 alleles known
- most are ER transport defective



ΔF508 = temp. sensitive

Glycerol
-can rescue many yeast Ts mutants
-probably by altering folding

Kevin Gunderson

- most ABC transporters transport large molecules

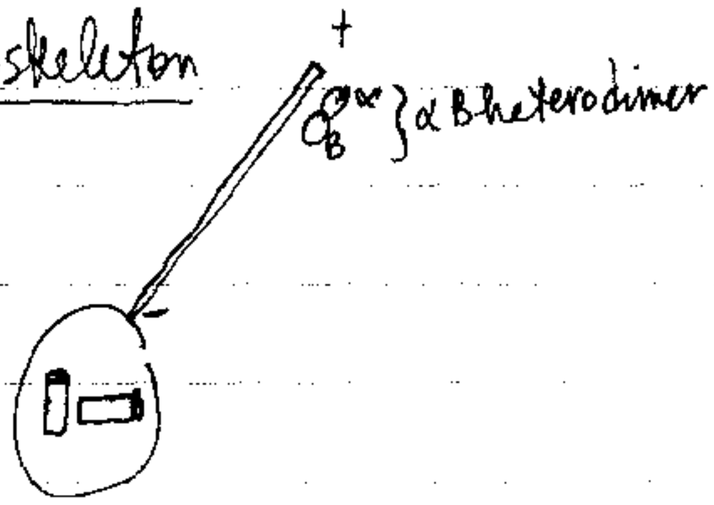
CFTR

- ATP opens channel
- appears to require ATP hydrolysis

what is evidence that opening required ATP hydrolysis

Bobby Jeng

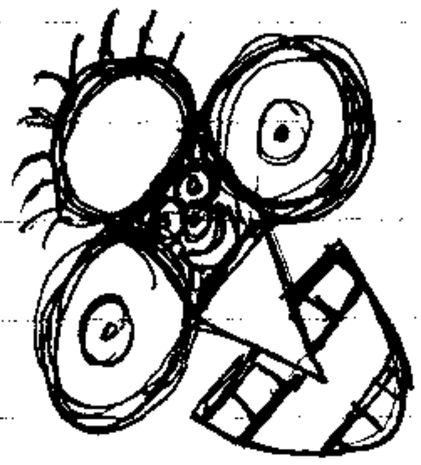
Microtubule cytoskeleton



- 3rd tubulin - first in Aspergillis - γ
  - γ localized only to MTOC (microtubule organizing centers)
  - cloned in many species

- S. cerevisiae - has γ-tubulin-like gene = TUB4

Sharon Long



- GroEL affects NOD function

Why legumes?
- some receptor

what about injecting nod factor into cells?