

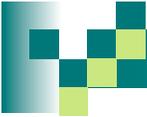
Creating and Presenting Dynamic Posters



www.training.nih.gov

Poster sessions are a lot like farmers markets





A poster is more than presenting data

- Develops your verbal communication skills
- Allows you to share information with the scientific community
- Builds networks & contacts
- Helps identify and establish collaborations
- Is a great source of feedback
- May even help you find a job



Effective poster presentations:

- are organized
- are visually appealing & readable
- are succinct
- presented clearly & with enthusiasm
- tell a story



And are not...

Manuscripts printed out and tacked up on a board!!!

REMEMBER:

- The average person decides within ~10 seconds whether to stay or go onto the next poster
- Most people stay at a poster less than 10 minutes



The most important rules

- Have your poster fit in the space provided
- Make your poster readable from 4 feet away
- Don't try to put **EVERYTHING** on your poster
- Limit text



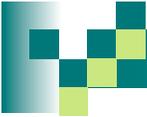
More tips for readable posters

- Use dark text on a light background
- Avoid garish colors and complicated backgrounds
- Use one font and style for the whole poster
- Leave space between columns
- Try to use bullet points and pictures instead of text
- Label figures clearly



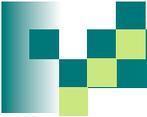
How large should the fonts be?

- Title 72 pt
- Section heading 48 pt
- Figure heading 30 pt
- General text 28 pt
- Text for labels 20 pt



Typical parts of a poster

- Title
- Authors and affiliations
- Introduction
- Goals (optional)
- Methods
- Results
- Conclusions
- Future directions (optional)
- References (optional)
- Acknowledgements



Short descriptive poster title

Authors & affiliations

Introduction
Goals
Methods

Data 1
Data 2
Data 3

Data 4
Conclusions
Other stuff

- Present data from top to bottom, left to right
- Maintain empty space between rows

Somatic journey to pluripotency and back to lineage commitment

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Background

Somatic cell reprogramming reverses the epigenetic and subsequently the differentiation identity of a cell to a pluripotent embryonic stem cell-like state. Embryonic stem cells (ESC), obtained from the inner cell mass of the blastocyst, are pluripotent: they are unspecialized, possess long term renewal ability and can give rise to the whole embryo excluding the extraembryonic tissue. As such they are highly prized for patient specific tissue replacement. The birth of Dolly in 1997, by somatic cell nuclear transfer, showed that: cellular differentiation is a reversible process when germ line modifications are not involved. Thus, in the presence of the appropriate "reprogramming environment" the epigenetic memory of a cell is re-established to a pluripotent-like state. A somatic cell becomes pluripotent-like when fused with an ESC either by polyethylene glycol (PEG) or by electrofusion. In 2006, Yamamaka *et al.* showed that this "reprogramming environment" can also consist of four retrovirally encapsulated transcription factor genes, which when transfected into somatic cells give rise to *induced pluripotent stem (iPS)* cells.



Dolly the sheep

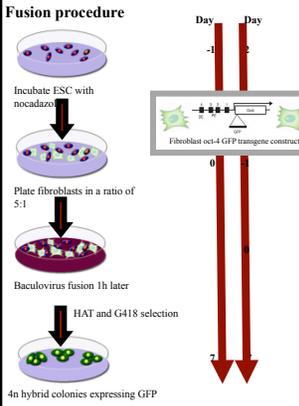
Objective

We have employed two strategies to investigate interrelated factors influencing somatic cell reprogramming:

- *Baculovirus mediated fusion* of two ESC lines with mouse embryonic fibroblasts (MEFs) investigating:
 1. Is the reprogramming ability of different ESC lines, as measured by the overall number of tetraploid hybrids obtained, "the same"?
 2. Are chromatin remodeling markers involved in modulating this phenotype and if so how?

Materials and Methods

Fusion procedure



iPS generation

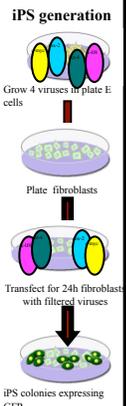
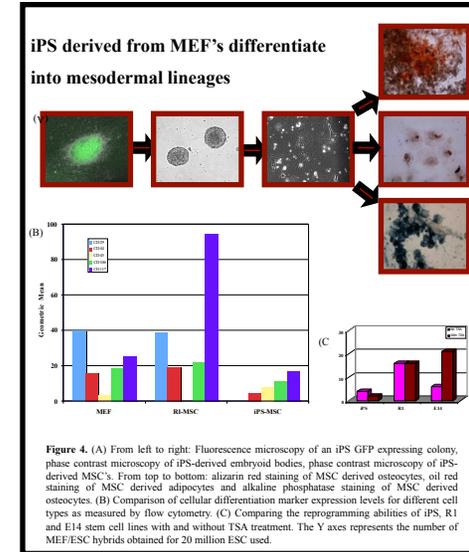
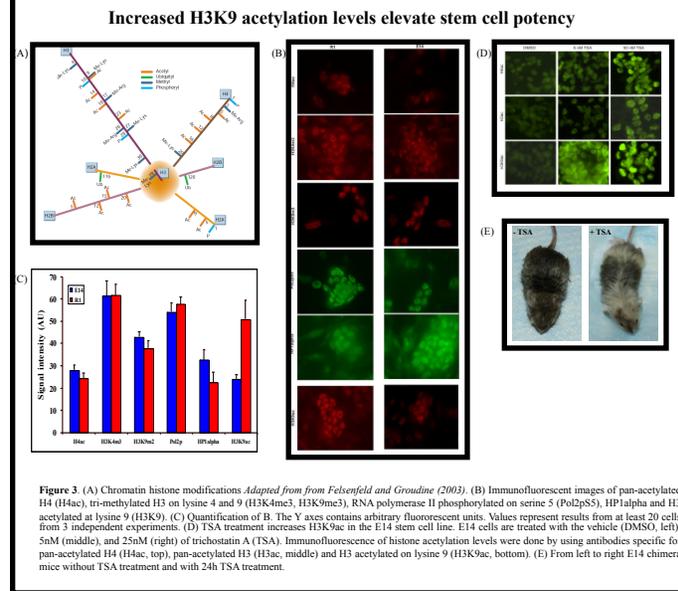
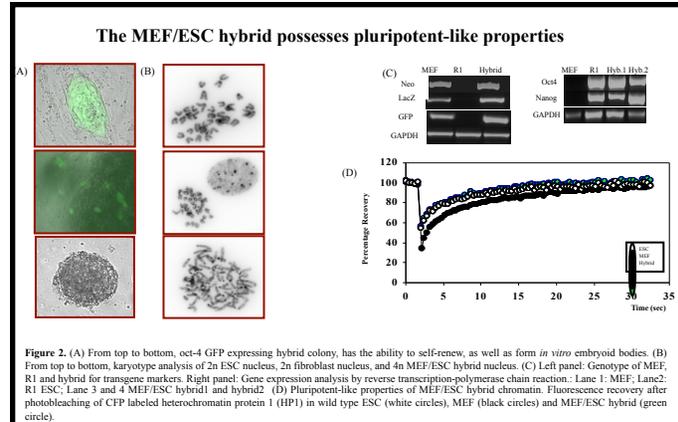


Figure 1. Flow chart of reprogramming by baculovirus mediate cell fusion (left panel) and by retroviral transfection of 4 genes (right panel).



Conclusion

- Reprogrammed hybrids exhibit pluripotent like characteristics such as morphology, long term renewal ability, embryoid body formation, gene expression profile and chromatin protein hyperdynamic plasticity.
- Different ESC lines display characteristic higher-order chromatin structure. While it is true that no one singular epigenetic modification invariably translates to one single biological output, we have shown that pharmacologically elevated levels of H3K9ac significantly increase the overall reprogramming ability of the E14 ESC line as measured by the most stringent reprogramming criterion: chimera contribution.
- When iPS are fused again with somatic cells from which they themselves originated, they reprogram them, although the efficiencies of this reprogramming merit further investigation.
- iPS differentiate into MSC's but flow cytometry analysis indicates that there are significant differences in the cellular differentiation marker levels as compared to standard *in vitro* MSC's.

Future

- iPS are heterogeneous with respect to pluripotency. In attempts to "quantify" such stemness differences we will investigate iPS chromatin epigenetic remodeling.
- The *in vivo* aspect of our work, will focus on examining the functional potential of iPS derived differentiated cells.
- Present iPS generating methods are such that these "golden cells" are still disqualified for translational use due to their increased oncogenic potential. We are working on finding new strategies to efficiently generate clinically usable iPS.

References

1. Conlay C, Nishida T, Miki H, et al. Nuclear reprogramming of somatic cells. *Science*. 2008; 319(5861):1771-1775.
2. Beckwith B, Krauss RS, Yoshida M, Herzig T, and Riggs T. Trichostatin A induces an epigenetic signature and global gene expression by blocking histone deacetylase in human carcinoma cells. *Epigenetics of cancer* 2:4, 109-122 (2008).
3. Gokhale S, Villalobos C, George S, Kessler JI, Brown DT and Mami T. Hippocampal plasticity of chromatin proteins in pluripotent embryonic stem cells. *Development* 135: 105-114 (2008).
4. Miki H, et al. Trichostatin A induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 131: 474-479 (2008).



The Title

- Your whole project boiled down to just a few words
- Used by many to decide whether to visit your poster
- Should not be too long or contain jargon and abbreviations
- Should state the main focus of your study; if it is too general, few people will come
- Must be visible from 6 feet away



Names and affiliations

- Include first and last names
- Spell out affiliations that may not be universally recognized
- Street addresses are not necessary
- Smaller than title, but still big
- Logos and pictures can be nice, but not if they clutter up your poster

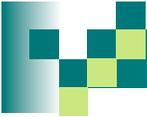


Introduction

- Gets the viewers interested and brings them up to speed in the field
- Puts your work into the context of what is known
- Justifies your model system and approach
- Often ends with a clear statement of your specific goals or hypothesis

REMEMBER:

- Keep it brief
- Use figures & diagrams if possible
- Use bullet points if possible

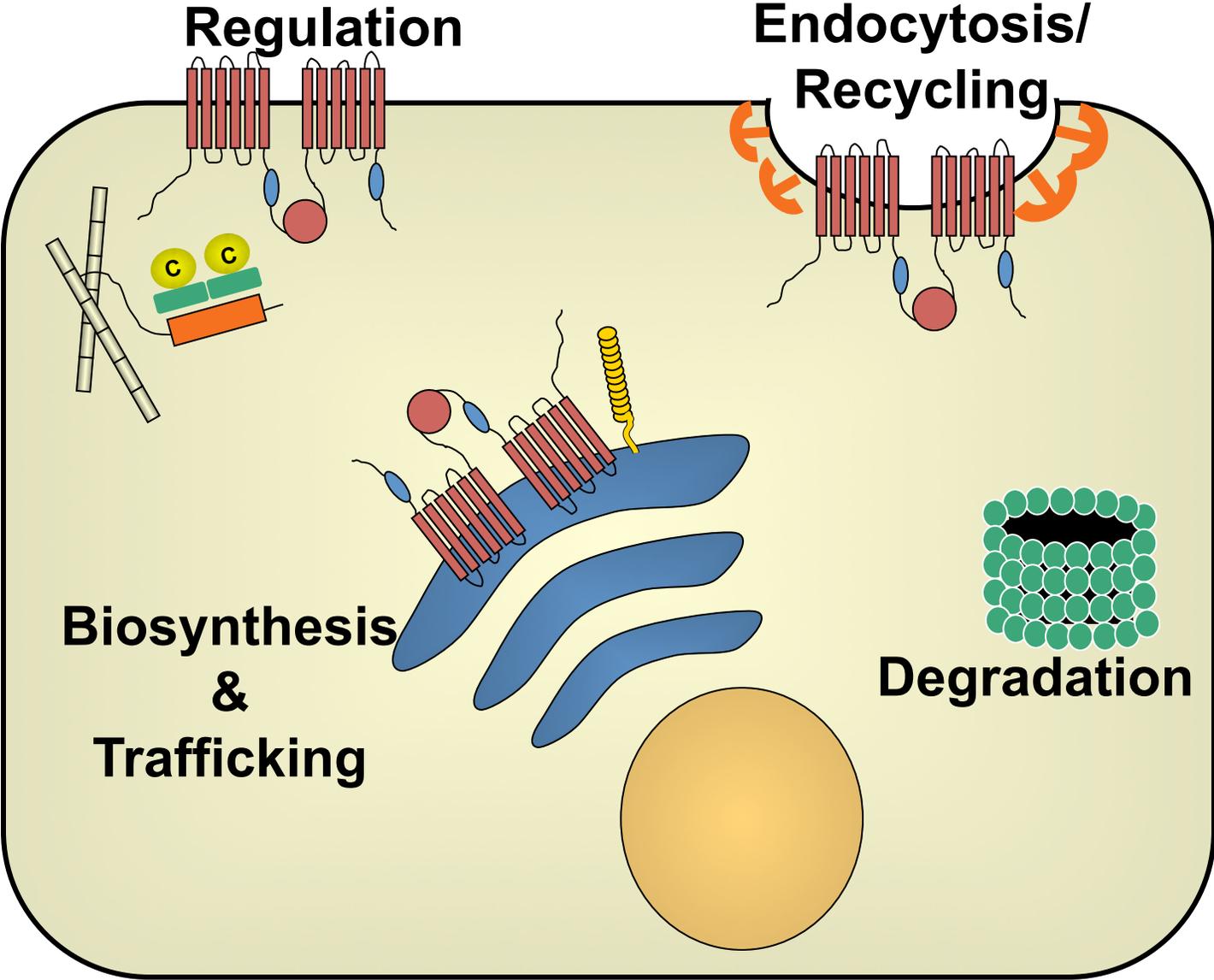


INTRODUCTION – a good example

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is an apical membrane Cl⁻ channel expressed in a variety of epithelial cells. CFTR is a member of the ABC transporter superfamily with two Nucleotide Binding Domains (NBD) and a large Regulatory (R) domain. Although key to understanding the mechanisms underlying cellular defects in CF, we still know little about cellular factors that regulate CFTR biosynthesis, trafficking, and regulation in polarized cells. Recent data suggests that CFTR exists as part of a multiprotein complex, but few CFTR-interacting proteins have been identified or characterized. Furthermore, we know little about how these interactions are modified in mutant proteins known to cause CF. We used proteomic approaches to identify novel CFTR-interacting proteins and characterized these interactions using a series of biochemical and cellular assays in cells expressing wild-type and mutant CFTR proteins.

6 sentences!

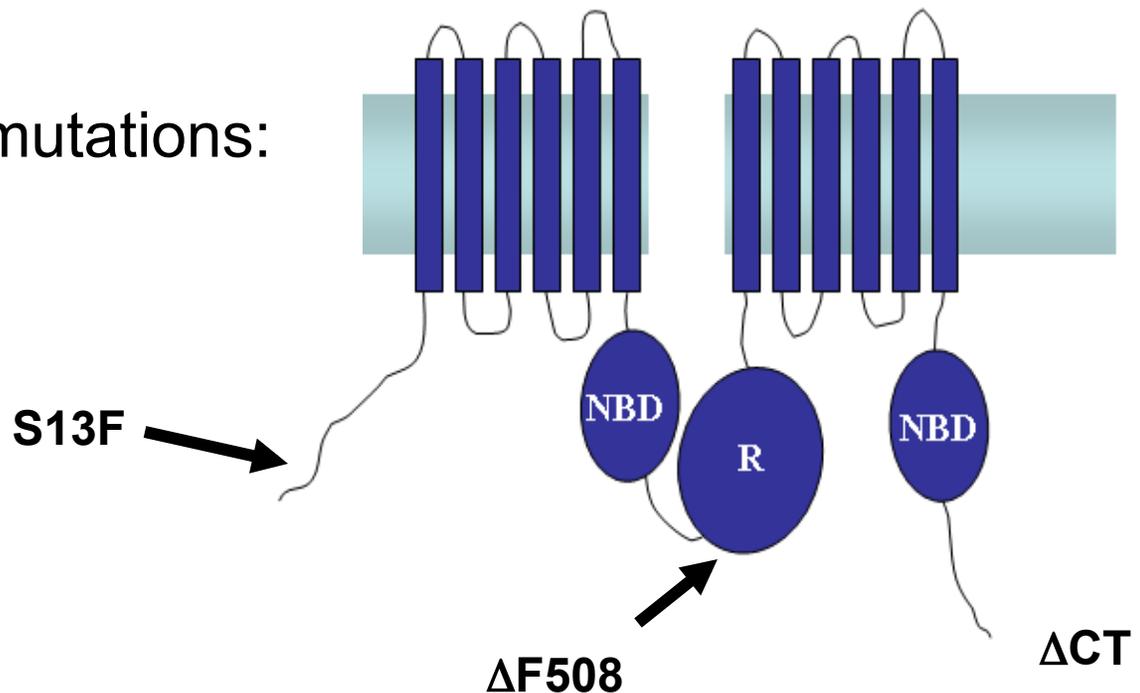
Protein interactions regulate all aspects of CFTR biology



Our Goals:

- To use mass spectrometry to identify proteins that associate with CFTR
- To use biochemical and cell-based assays to study novel CFTR-interacting proteins
- To study novel interacting proteins in cells expressing WT and mutant versions of CFTR

We focused on 3 mutations:
N-terminal, S13F
R domain, Δ F508
C-terminal, Δ CT





Methods

- As brief as possible
- Use graphics and flow charts, rather than text, if possible
- No need to describe basic methods

REMEMBER:

Most viewers don't want to read the details; they will ask for details if they want them



Our overall approach:

**USE MS TO IDENTIFY
INTERACTORS**

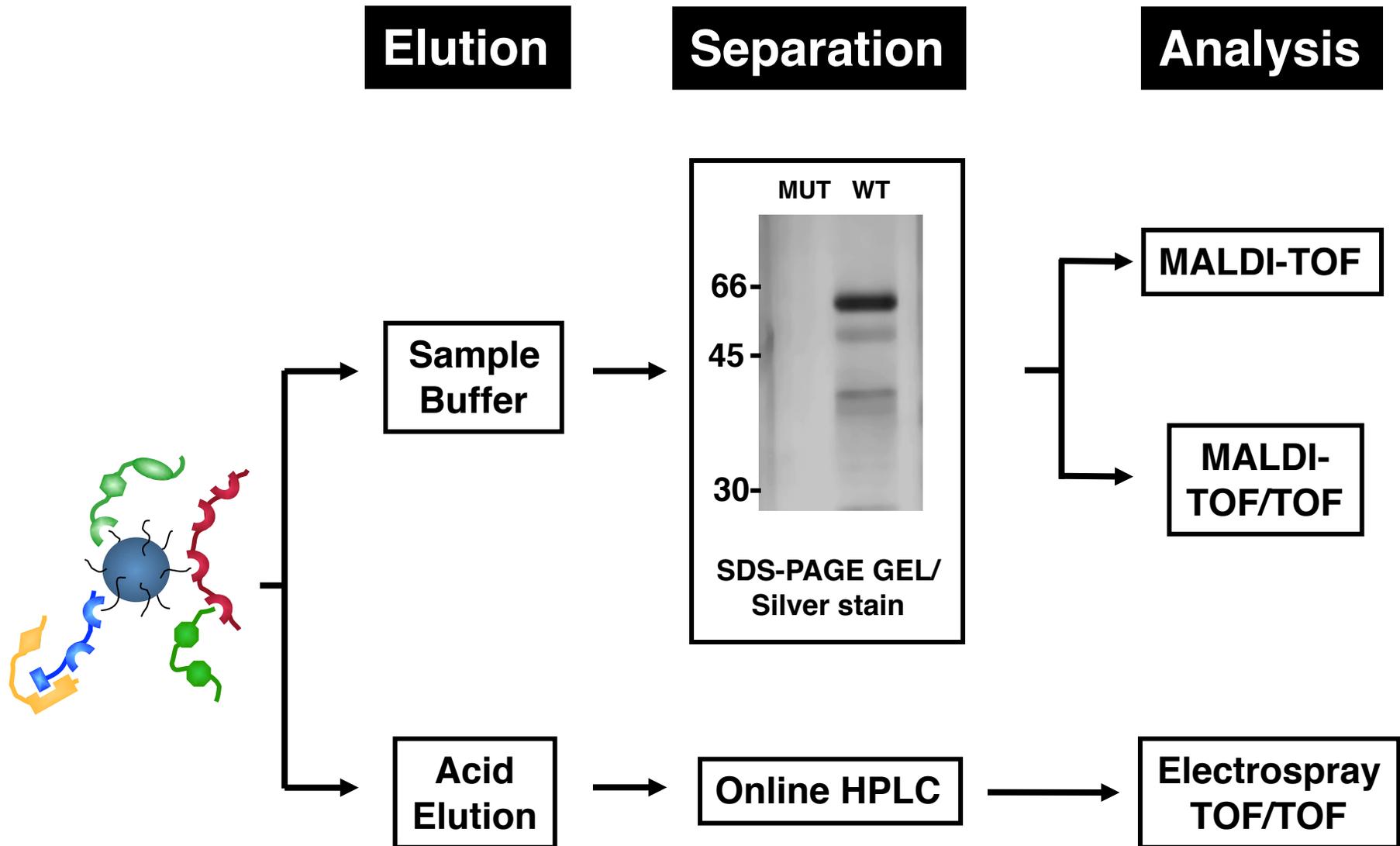


**VALIDATE USING
BIOCHEMICAL APPROACHES**



**CHARACTERIZE USING FUNCTIONAL
ASSAYS**

Sample prep and analysis





Results

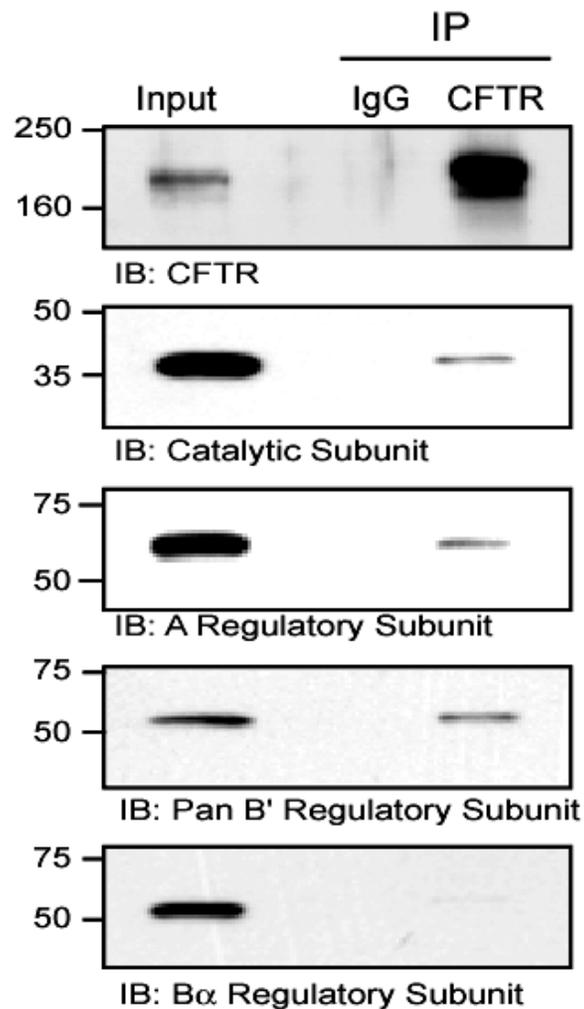
- Include only a few key figures or tables
- Each figure should have a title that summarizes the results
- Figures should be large, labeled clearly and be easy to understand without a long legend
- All text should be visible from several feet away

REMEMBER:

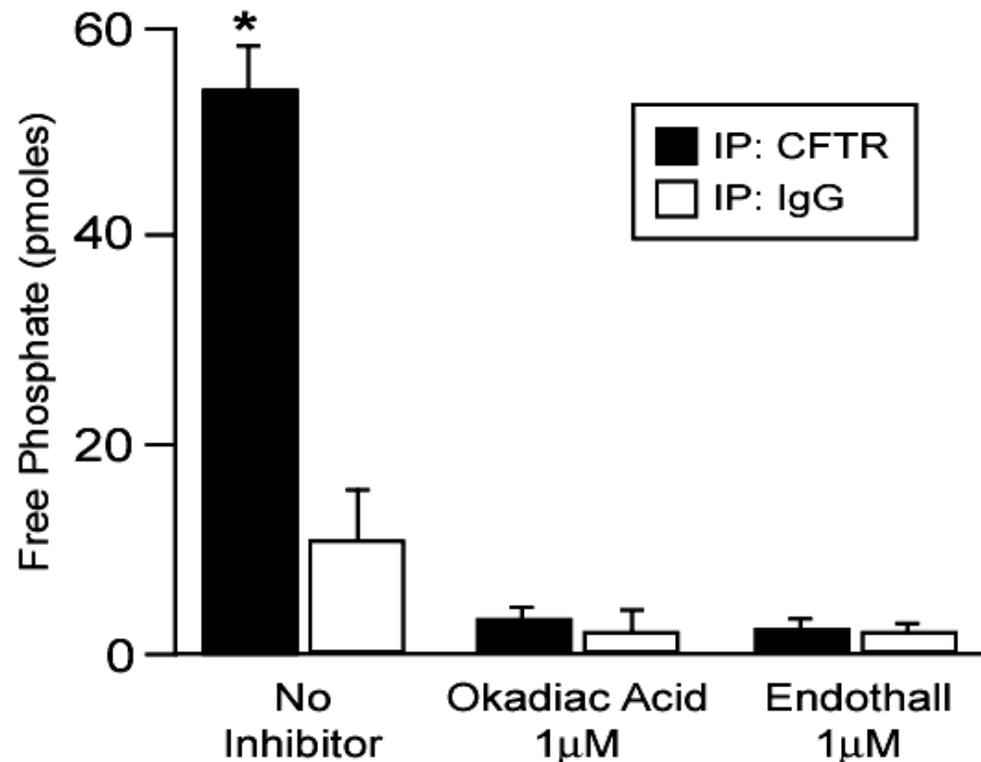
- Figures should make sense even when you're not at your poster
- Put your best foot forward!

PP2A subunits & activity co-precipitate with CFTR in airway cells

A. Western blot analysis

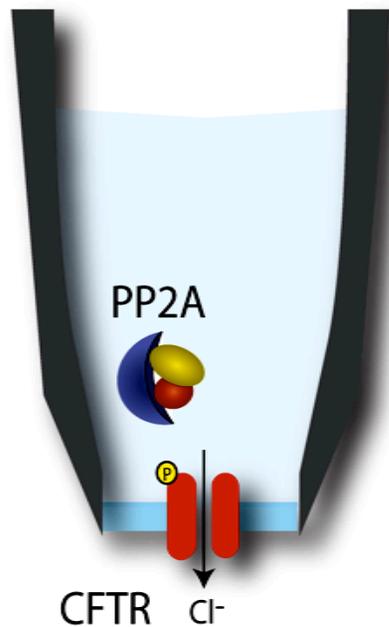


B. Phosphatase activity assay

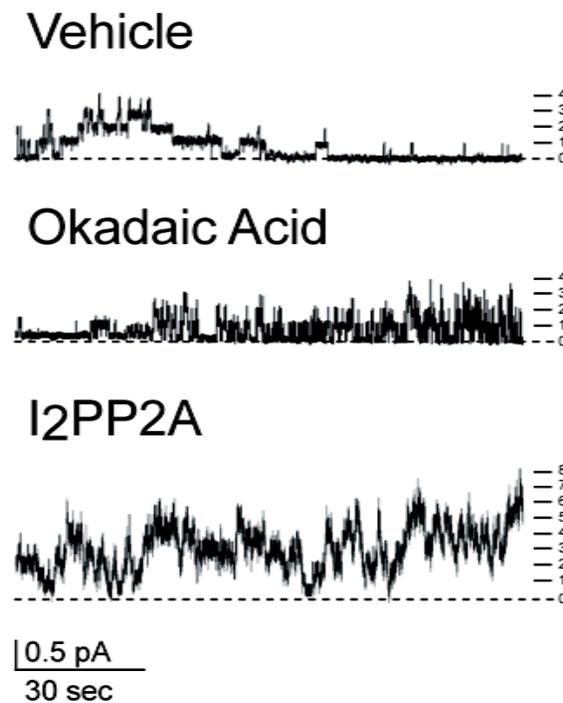


PP2A regulates CFTR channel activity

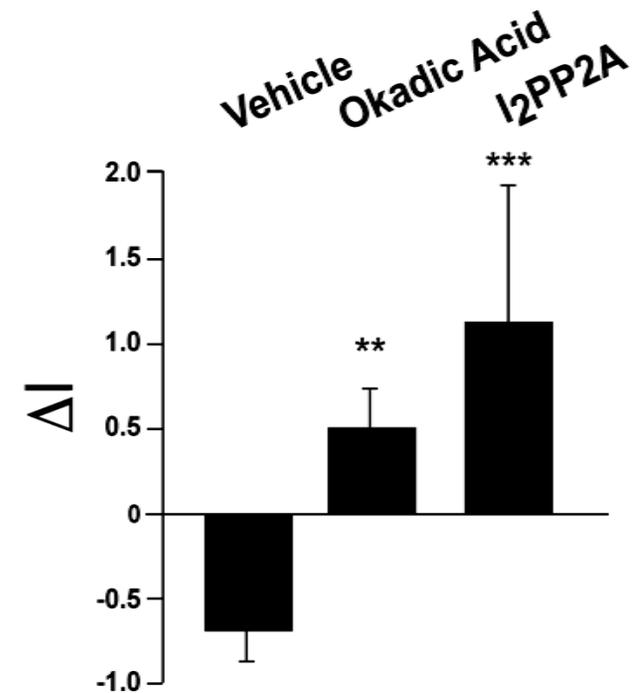
A. Experimental design



B. Single channel recordings



C. Averaged data
N=6





Conclusions

- Use bullet points to highlight major findings
- Consider displaying a model
- Possible to use summary paragraph or summary bullet points instead

REMEMBER:

Less is more!



CONCLUSIONS –

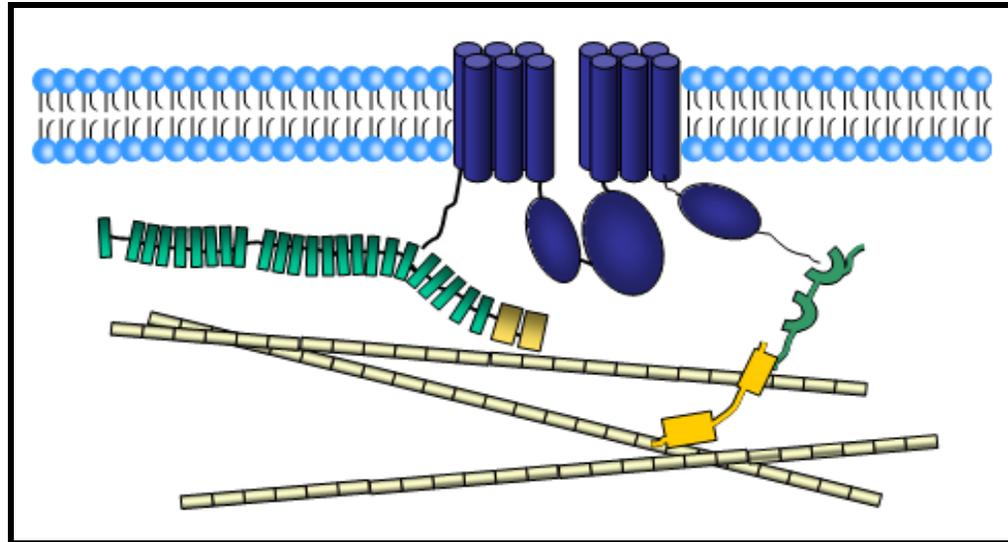
We used affinity purification to identify proteins that associate with CFTR and found that the the B' ϵ subunit of PP2A directly associates with the CFTR C-terminus. Using western blotting and in-vitro phosphorylation assays, we showed that PP2A protein and activity co-immunoprecipitate with CFTR from airway epithelial cells. The PP2A B' ϵ is the subunit responsible for targeting the phosphatase to the channel. We further found that PP2A negatively regulates CFTR channel activity in mouse intestinal and human airway epithelial cells. Thus we conclude that inhibitors of PP2A may improve clinical outcomes in cystic fibrosis.



Isn't this better?

- The B' ϵ subunit of PP2A directly associates with the COOH-terminus of CFTR
- PP2A protein and activity co-immunoprecipitate with CFTR in cultured airway epithelial cells
- PP2A negatively regulates CFTR channel activity in mouse intestinal and human airway epithelial cells
- Inhibitors of PP2A may improve clinical outcomes in cystic fibrosis

Filamins may regulate many aspects of CFTR function



Our data suggest three possible functions :

- Stability on the cell surface
- Scaffolding regulatory factors
- Directly altering channel activity



Future Directions

- Optional section
- Use bullet points
- Be brief

Targeting Human Disease with Virus Mimicry

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¹Center for Cancer Research Nanobiology Program, National Cancer Institute at Frederick, National Institutes of Health, Frederick, MD

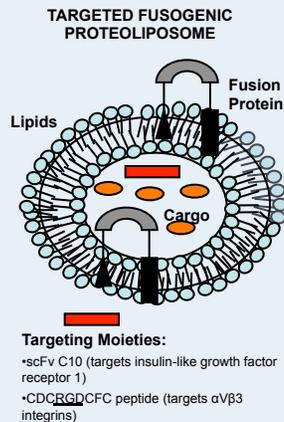
²Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, State University of New York (SUNY) at Buffalo, Buffalo, NY

³SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD

Abstract

Viruses hijack human cells using a variety of sophisticated mechanisms that range from fusion with the cell membrane to regulation of protein expression and genetic modification. These natural principles are excellent models from which we can design targeted therapies to treat human disease.

We are designing nanoparticles that are based upon virus entry mechanisms. One of our hypotheses is that the efficiency of nanoparticle payload delivery can be dramatically enhanced by the capacity for direct membrane fusion with the plasma membrane. We are utilizing viral membrane fusion proteins incorporated into liposomal nanoparticles to deliver payloads directly into the cytoplasm of targeted cells.



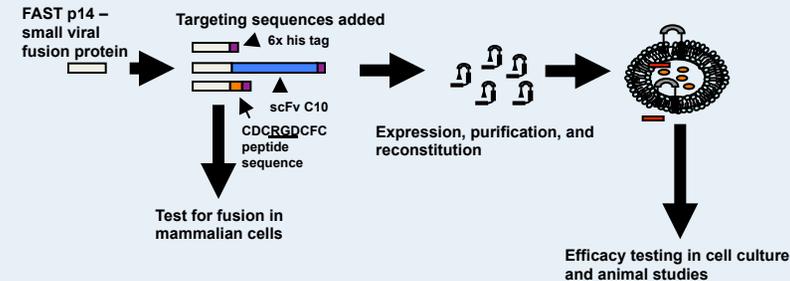
Introduction

The great promise of **nanoparticle delivery** is its ability to salvage drugs or other therapy modalities that have successfully made it far into preclinical or clinical trials, but that have failed near the end of the pipeline because of toxicity or deleterious immunological response.

Liposomes present a promising biomaterial-based method of therapeutic delivery, constituting more than 250 NIH clinical trials.¹ A primary issue that remains unresolved in liposomal delivery, and in nanoparticle delivery in general, is avoidance of the endocytic pathway, which often leads to uncontrolled release, sequestering, and/or degradation of cargo molecules in vesicles in the entry pathway.

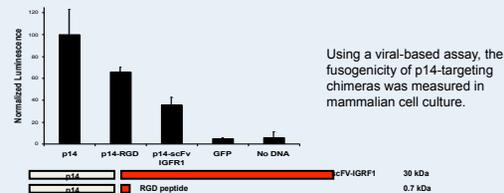
Our goal is to avoid the endocytic pathway by direct fusion with the plasma membrane. The fusogenic protein that we use is a fusion-associated small transmembrane (FAST) protein, p14, from a reptilian reovirus.² FAST p14 is promising in engineering fusogenic liposomes because it is much smaller, at 14 kD, and less complex than other fusogenic protein machinery, for instance, the HIV-entry machinery, which is a trimer of heterodimers at ~500 kD.

Methodology



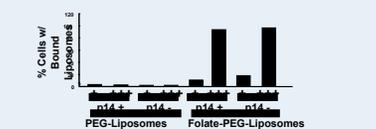
Results

Fig. 1: FAST p14 chimeras containing C-terminal targeting peptides retain fusogenic activity.



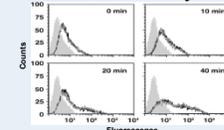
Using a viral-based assay, the fusogenicity of p14-targeting chimeras was measured in mammalian cell culture.

Fig. 2: FAST p14 liposomes can be targeted to specific cell receptors.



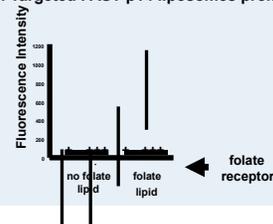
Treatment of cells to up-regulate the folate receptor resulted in a dramatic increase in liposome adherence to target cells.

Fig. 3: FAST p14 liposomes promote fusion and intracellular delivery.



The increased fluorescence in cells seen by the rightward shift in fluorescence, indicates that calcein entrapped in the liposomes, self-quenching at higher concentrations, has been released into the cytoplasm.

Fig. 4: Targeted FAST p14 liposomes promote increased intracellular delivery.



Cell fluorescence increase caused by folate-targeted liposomal delivery was quantified, correcting for the background fluorescence at the non-fusogenic temperature of 4°C.

Conclusions

- FAST p14 remains fusogenic with the addition of targeting moieties to the C-terminus of the protein.
- FAST p14 does not interfere with targeting of liposomes to cells using a folate lipid targeting the folate receptor.
- Targeted-FAST p14 liposomes show increased intracellular delivery.

Ongoing Work

- Test RGD and scFV-chimeras for targeted fusion with cells
- Encapsulate and deliver cytotoxic drugs
- Encapsulate and deliver pro-apoptotic peptides
- Deliver DNA/RNA
- Begin testing in small animal models

Future plan

Pursue detailed studies of virus mechanisms with an eye toward utilization of this knowledge to drive innovation in nanomedicine.



References

1. Information on Clinical Trials. *National Library of Medicine*. www.clinicaltrials.gov.
2. Top, D, R de Antueno, J Salsman, J Corcoran, J Mader, D Hoskin, A Touhami, MH Jericho, R Duncan (2005). *EMBO J*. 24: 2980-2988.

Collaborators

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Dimitar Dimitrov, Center for Cancer Research Nanobiology Program, NCI-Frederick, Frederick, MD

SAIC SAIC-Frederick, Inc. A subsidiary of Science Applications International Corporation

A relationship between yeast nuclear morphology, large structural nucleoporins, and the SUN domain protein *MPS3*

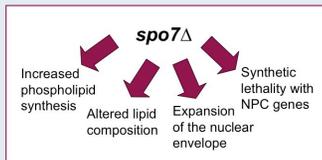
Keren L Witkin¹, Sue Jaspersen² and Orna Cohen-Fix¹

¹NIDDK, National Institutes of Health, Bethesda, Maryland 20892.

²Stowers Institute for Medical Research, Kansas City, MO 64110.

Abstract:

Cells lacking the non-essential *SPO7* gene have increased phospholipid synthesis, altered lipid composition, and expansion of the nuclear envelope. *SPO7* has genetic interactions with genes encoding nuclear pore complex (NPC) components, suggesting that NPCs are affected by the altered nuclear membrane in *spo7Δ* cells. In order to identify other nuclear envelope components sensitive to *SPO7* deletion, we adopted a candidate approach. One interesting candidate is the SUN-domain gene *MPS3*. The Mps3p protein localizes to the spindle pole body (SPB) and around the nuclear periphery, and *MPS3* has been implicated in SPB duplication as well as other nuclear functions. Cells lacking *SPO7* were compromised for viability when combined with *mps3* mutations specifically affecting Mps3 function at the SPB. This suggests that *SPO7* deletion exacerbates the SPB defect of *mps3* mutants, rather than affecting its other nuclear functions. High-copy suppressors of the synthetic lethality between *spo7Δ* and *mps3-1* were identified, including dominant-negative forms of the nucleoporins *NUP157* and *NIC96*. Strikingly, deletion of *NUP157* completely rescues the SPB defect of *mps3* mutants. Furthermore, deletion of *NUP157* or either of the pore membrane (pom) nucleoporins *POM152* or *POM34* rescues temperature-sensitivity of a subset of SPB mutations, including *mps3*. Based on our data, we propose 3 models for the relationship between nucleoporins, *MPS3* and the SPB.



Working hypothesis:

Altered nuclear envelope lipid composition in *spo7Δ* cells affects the function of proteins and complexes that reside in the nuclear membrane.

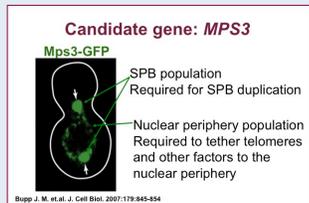


Figure 1: Genetic interactions between *SPO7* and *MPS3*

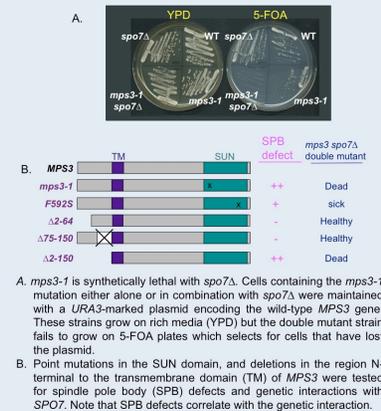
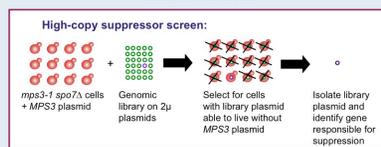


Figure 2: Genetic interactions between *SPO7* and other SPB genes

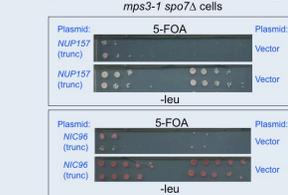
SPB alleles synthetically sick or lethal with <i>spo7Δ</i>	SPB alleles with no apparent genetic interaction with <i>spo7Δ</i>
<i>mps2-381</i>	<i>mps2-1</i>
<i>kar1Δ17</i>	<i>cdc31-2</i>
<i>ndc1-39</i>	<i>spc110-220</i>
<i>spc29-3</i>	<i>mps1-1</i>
<i>spc42-11</i>	

Figure 3: Suppressor screen schematic and results:



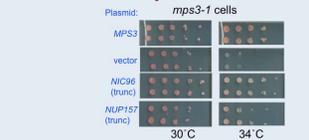
Gene	Function
<i>NBP1</i>	SPB duplication
<i>SLT2</i>	MAP kinase in the cell integrity pathway. Implicated in SPB duplication.
<i>NUP157</i> (truncated)	Large structural nucleoporin
<i>NIC96</i> (truncated)	Large structural nucleoporin
<i>PSA1</i>	GDP-mannose pyrophosphorylase involved in cell wall structure

Figure 4: Truncated forms of *NUP157* and *NIC96* partially suppress synthetic lethality



mps3-1 spo7Δ cells carrying a *URA3*-marked *MPS3* plasmid were transformed with *LEU2* plasmids containing truncated forms of *NUP157* (top box) or *NIC96* (bottom box), and then tested for growth in the presence (-leu) and absence (5-FOA) of the *MPS3* plasmid. Note that both suppressor clones partially restored growth on 5-FOA media, which requires growth in the absence of the *MPS3* plasmid. Full-length forms of *NUP157* and *NIC96* failed to suppress synthetic lethality (not shown).

Figure 5: Both suppressors rescue ts of *mps3* mutants



mps3-1 cells were transformed with the indicated *LEU2* plasmids and tested for growth at 30°C and 34°C. Note that truncated forms of *NUP157* or *NIC96* restored growth at 34°C. Full-length forms of *NUP157* and *NIC96* failed to suppress temperature-sensitivity (not shown).

Figure 6: *NUP157* or pom deletions rescue ts of some SPB mutants

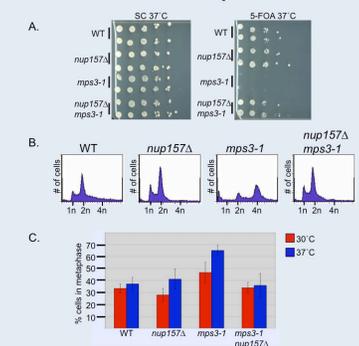
SPB allele	Suppression by <i>nup157Δ</i>	Suppression by <i>pom152Δ</i>	Suppression by <i>pom34Δ</i>
<i>mps3-1</i>	++	++	++
<i>mps2-381</i>	++	++	++
<i>mps2-1</i>	-	++	++
<i>spc42-11</i>	+	-	-
<i>spc98-2</i>	-	TBD	TBD
<i>spc29-3</i>	-	TBD	TBD
<i>mps3Δ</i>	TBD	TBD	TBD

++ Strong suppression at 37°C + Weak suppression at 37°C
- No suppression at 37°C TBD To be determined

Models:

- Nucleoporins affect the relative localization of Mps3 to the nuclear envelope vs the SPB. In the absence of *NUP157*, Mps3 preferentially localizes to the SPB, and this rescues Mps3 function at the SPB.
- Nuclear pore complexes and the SPB compete for insertion into the nuclear envelope, perhaps because of a shared insertion factor. When NPC biogenesis is compromised, defects stemming from improper SPB duplication or insertion are ameliorated.
- Nuclear pore complexes affect nuclear membrane dynamics, which affects SPB duplication or insertion.

Figure 7: *NUP157* deletion rescues the SPB defect of *mps3* mutants



A. Cells with the indicated genotype containing a *URA3*-marked *MPS3* plasmid were plated by serial dilution on non-selective media (SC) and 5-FOA media which only allows growth in the absence of the plasmid. Note that deleting *NUP157* restored growth at 37°C to *mps3-1* cells.

B. Cells of the indicated genotypes were assayed for DNA content by flow cytometry. Note that the diploidization phenotype of *mps3-1* cells, indicative of an SPB defect, was rescued by deletion of *NUP157*.

C. Asynchronous cells in mid-log phase were maintained at 30°C or shifted to 37°C for 3 hours and then fixed with ethanol. Cell cycle distribution was determined by microscopy of DAPI-stained cells. Note that the metaphase delay seen in *mps3-1* cells was rescued by *NUP157* deletion.

Summary

• There are strong genetic interactions between *SPO7* and *MPS3*, specifically affecting *MPS3* function at the SPB.

• *SPO7* also affects viability of other SPB mutants, suggesting that *SPO7* influences SPB structure or function.

• High-copy expression of truncated forms of *NUP157* and *NIC96* rescues synthetic lethality between *spo7Δ* and *mps3-1* and partially rescues the temperature-sensitivity of *mps3-1* mutants.

• Deletion of *NUP157*, *POM152*, or *POM34* rescues the temperature-sensitivity of *mps3* mutants and a subset of other SPB mutants.

• Deletion of *NUP157* restores SPB function to *mps3-1* mutants, establishing a functional link between nuclear pore proteins and the SPB.



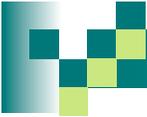
Poster Formats

- It is important to talk in advance with your mentor about options for poster design and printing
- Examples of format options:
 - One large page printed on a poster printer
 - Individual panels printed on 8 ½ x 11 paper and mounted on colorful poster board



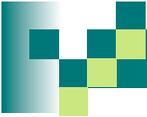
Before you print

- Discuss options with your mentor way ahead of time-you may need an appointment
- Print out a draft in 8 ½ x 11 format to proofread and edit
- Make sure you're using standard fonts and high quality images
- Think about saving a pdf version (this is especially useful if switching from a Mac to PC)



If you are using a graphics arts service

- Inform the service what program and platform you used to prepare your poster
- Make sure the service offers electronic approvals
- When checking your proof, make certain to read the text carefully
- Make sure you understand screen vs printing color differences



Common mistakes

- Too much material
- Too much text
- Poor layout
- Blocks of text longer than 10 sentences
- Waiting until last minute to print
- Neglecting to prepare your presentation



Useful Rules of Thumb

- A poster should be ~40% graphics, 20% text, and 40% empty space
- It takes 1 - 2 weeks to put together an outstanding poster



Making a terrific poster is only the first step

- How you present yourself and discuss your work is critical
- Wear a name tag & introduce yourself
- Practice! People will wander away if you are long-winded and hard to follow
- Listen carefully to questions; be prepared to provide detail for experts and an overview for interested novices
- If necessary, bring additional data, reprints, business cards, etc



New this year!

This year all posters will be judged by NIH scientists who will be looking at the following:

- **Poster Content**
- **Poster Appearance**
- **Student Presentation**