1. **Pick up** Name Folder

- Pick up name folder and set it up at seat.

2. **Sit** with your lab group.

- laptops almost closed (avoid distracting)

3. **Clicker** Attendance

- Launch your Top Hat, and get ready to click.

Budgeting homework time (60 min): Chapter 8, section 8.2 (first half) is 2498 words in length with four data tables and figures that require thinking and notetaking for the Trifecta. Reading at 200 words per minute would mean the section might take 13 minutes to read. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 60 minutes.

1. _____ **For Thursday's lecture**, read **Chapter 8: Cell Structure and Function**, section 8.2: "Why aren't there giant cells?" and as you read it be sure to take handwritten notes on the first half (2498 words). You can stop taking detailed notes when you begin reading the light blue box denoting the second section on "Calculated cell limitations". Just read that short section for deeper understanding.

2. _____ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _____ (Trifecta): **Prepare to explain (aloud) Figures 8.14, 8.15 and 8.16 in class** (Purpose, Methods, Findings)

4. _____ **Advanced**: Take a peek at some of the published research papers in the Bibliography at the bottom of the page with the goal to find the original figure you studied here in the reading and where it is in the paper?

Cell Size and Scale

Meter	Centimeter	Millimeter	Micrometer	Nanometer	Angstrom	Picometer
10^{0} m	10^{-2} m	10^{-3} m	10^{-6} m	10^{-9} m	10^{-10} m	10^{-12} m
1 m	0.01 m	0.001 m	0.000001 m	0.000000001 m	0.0000000001 m	0.000000000001 m
	1/100 m	1/1,000 m	1/1,000,000 m	1/1,000,000,000 m	1/10,000,000,000 m	1/1,000,000,000,000 m
	hundreth of a meter	thousandth of a meter	millionth of a meter	billionth of a meter	ten billionth of a meter	trillionth of a meter

https://learn.genetics.utah.edu/content/cells/scale/

trifecta+

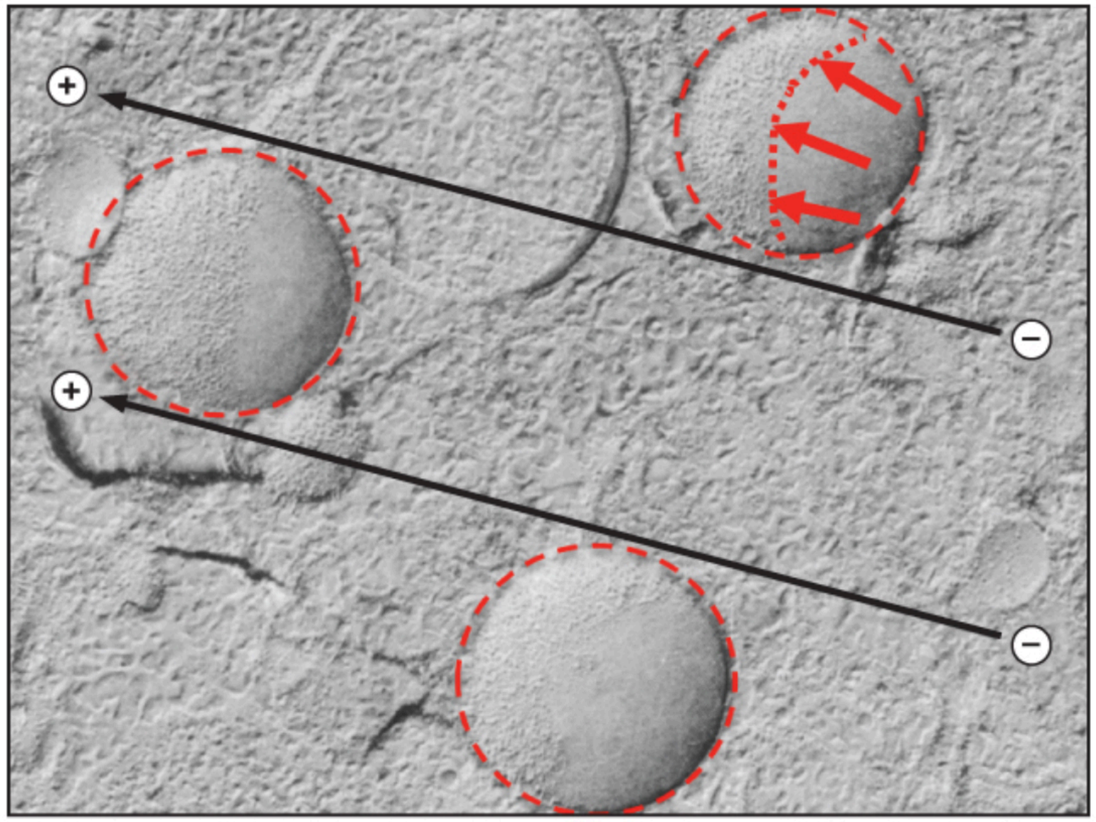

Figure 8.14 Mobility of membrane proteins. Three cells are outlined with red dashed lines. The electrical field is represented by black lines with the indicated polarity. In the top right cell, the movement of proteins is indicated with red arrows, and the edge of the accumulated proteins is indicated with a dotted arc. From Sowers *et al.,* 1981, modified from their figure 6. Sowers, Arthur E. and Charles R. Hackenbrock. 1981. Rate of lateral diffusion of intramembrane particles: Measurement by electrophoretic displacement and rerandomization. PNAS. Vol. 78(10): 6246-6250.

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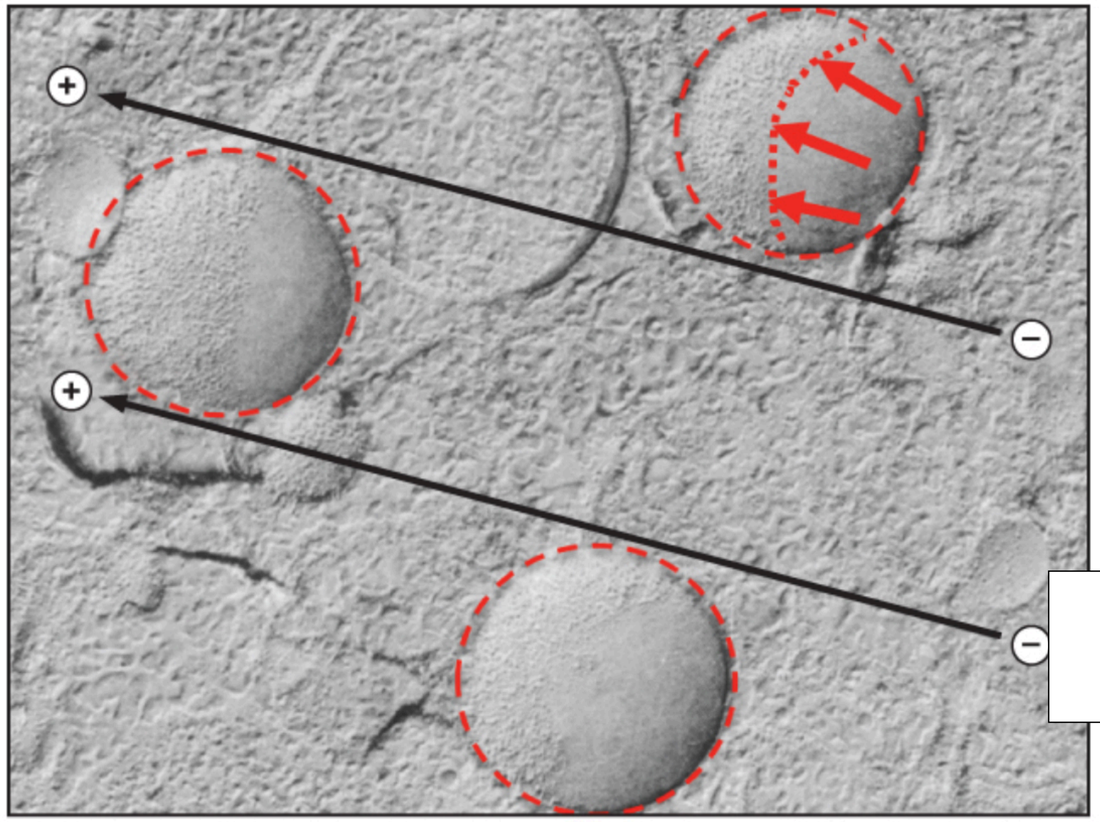
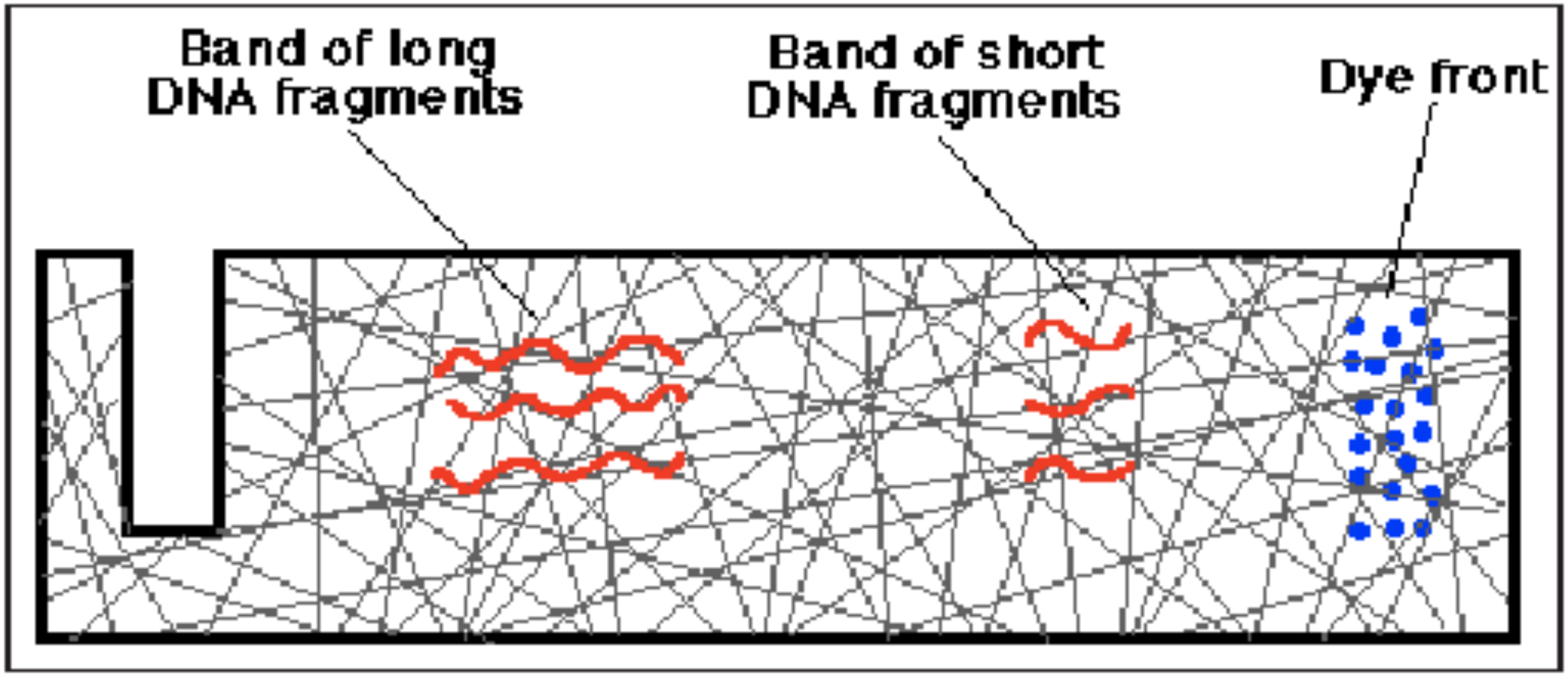


Figure 8.14 Mobility of membrane proteins. Three cells are outlined with red dashed lines. The electrical field is represented by black lines with the indicated polarity. In the top right cell, the movement of proteins is indicated with red arrows, and the edge of the accumulated proteins is indicated with a dotted arc. From Sowers *et al.,* 1981, modified from their figure 6. Sowers, Arthur E. and Charles R. Hackenbrock. 1981. Rate of lateral diffusion of intramembrane particles: Measurement by electrophoretic displacement and rerandomization. PNAS. Vol. 78(10): 6246-6250.

HYPOTHESIS?

Draw what the **Hypothesis** was

("Show and Tell" on projector)



HYPOTHESIS (agarose gel electrophoresis)

THE YAKU-BONCZYK PRIMER DESIGN

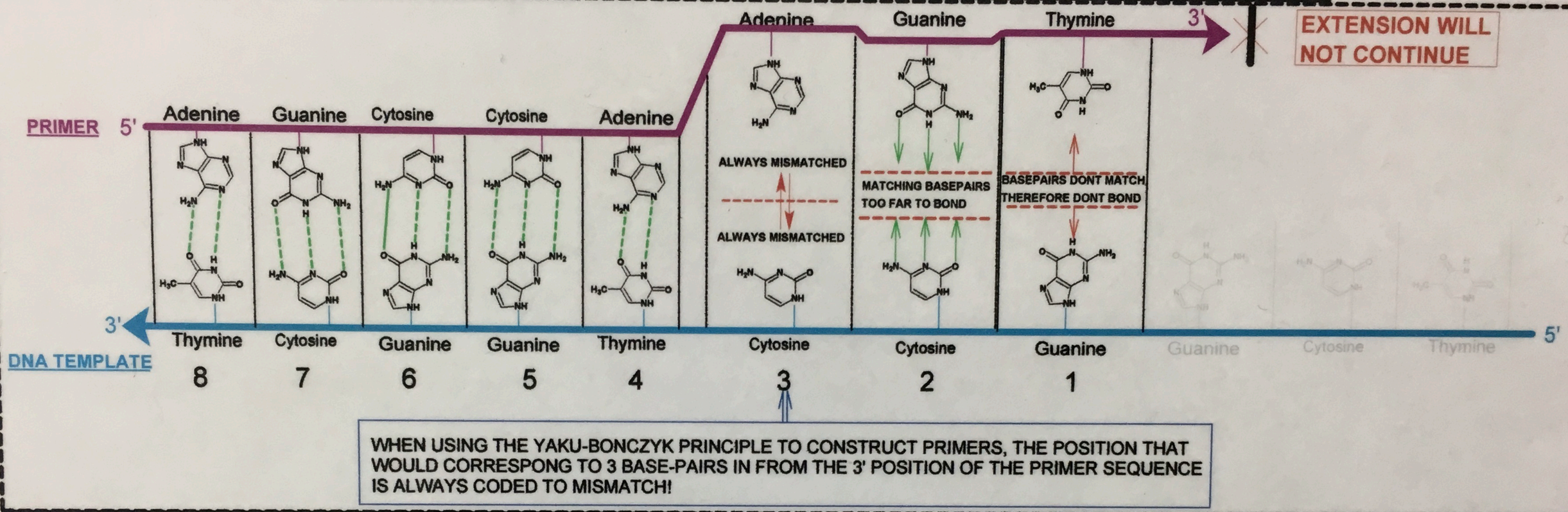


FIGURE 1: MUTANT-SEEKING PRIMER FOR WILD-TYPE DNA

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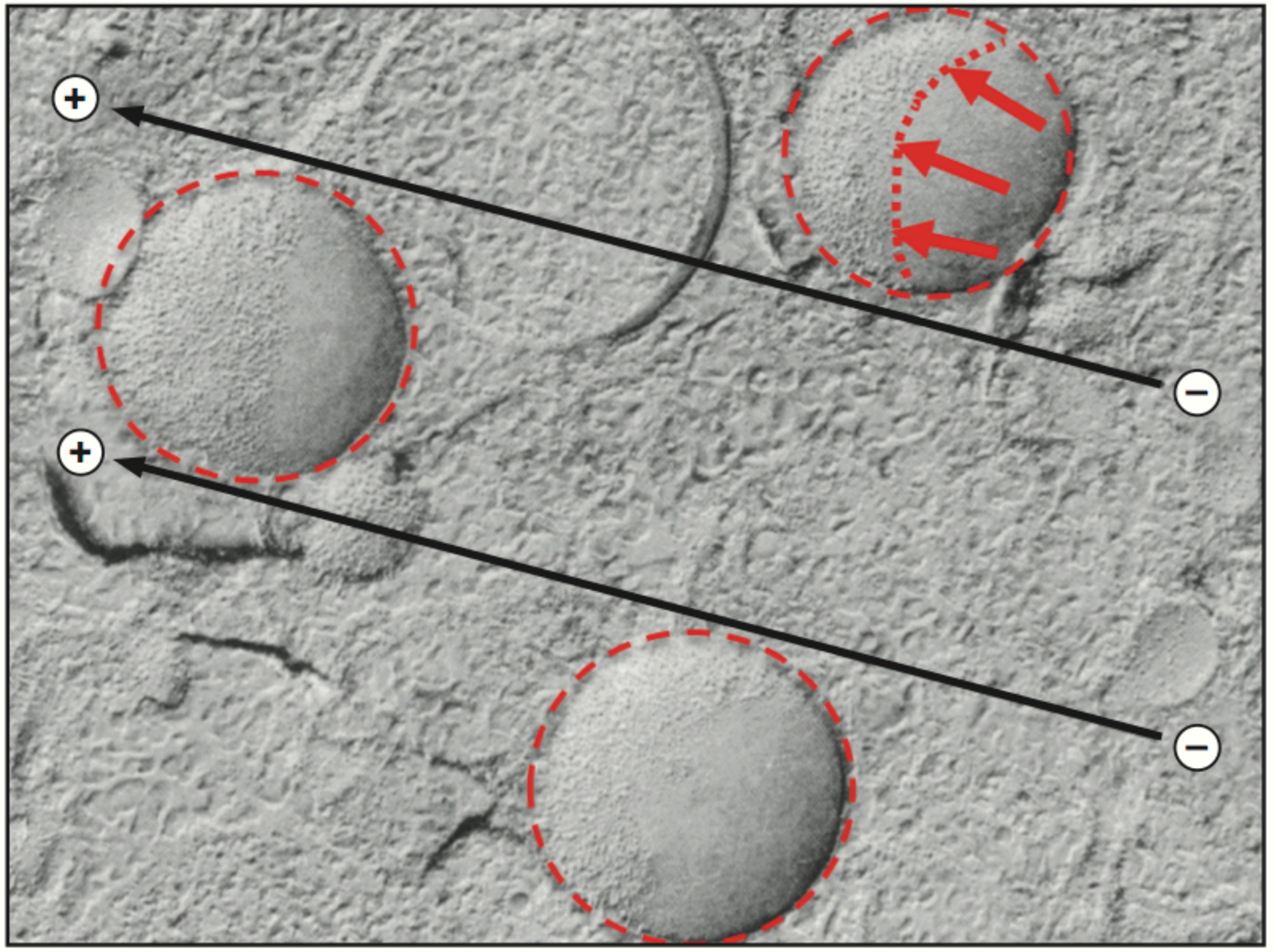


Fig. 8.14

modified from Sowers *et al.*, 1981

Methods (more details)

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6246–6250, October 1981
Cell Biology

Rate of lateral diffusion of intramembrane particles: Measurement by electrophoretic displacement and rerandomization

(lateral diffusion coefficient/integral proteins/freeze-fracture

ARTHUR E. SOWERS AND CHARLES R. HACKENBRO

Laboratories for Cell Biology, Department of Anatomy, School of Medicine, U

Communicated by Walther Stoeckenius, June 8, 1981

ABSTRACT A method combining electrophoresis and freeze
fracture electron microscopy is described; the method was use
to determine the lateral diffusion coefficient of intramembran

mean-square displacement of 57 nm in 10 msec is predicted.

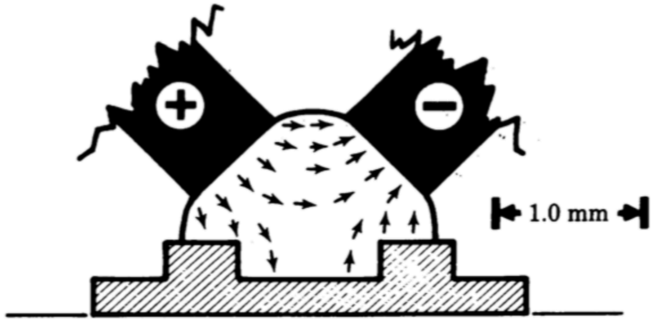


FIG. 1. Relationship of membrane microsuspension of a standard Balzers gold freeze-fracture specimen holder to Ag/AgCl wire electrodes and the expected electric field as represented by vectors.

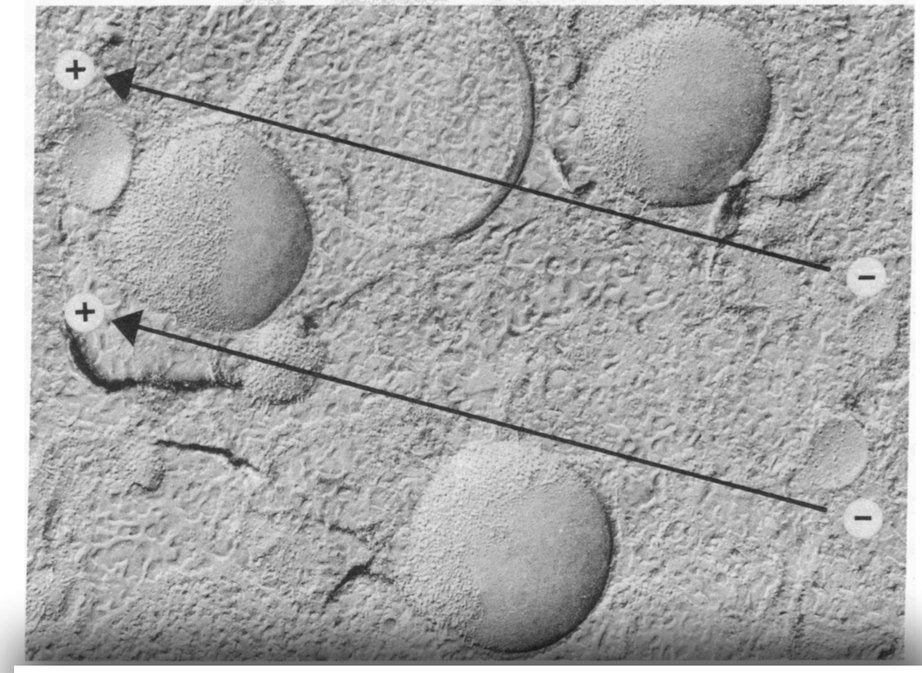
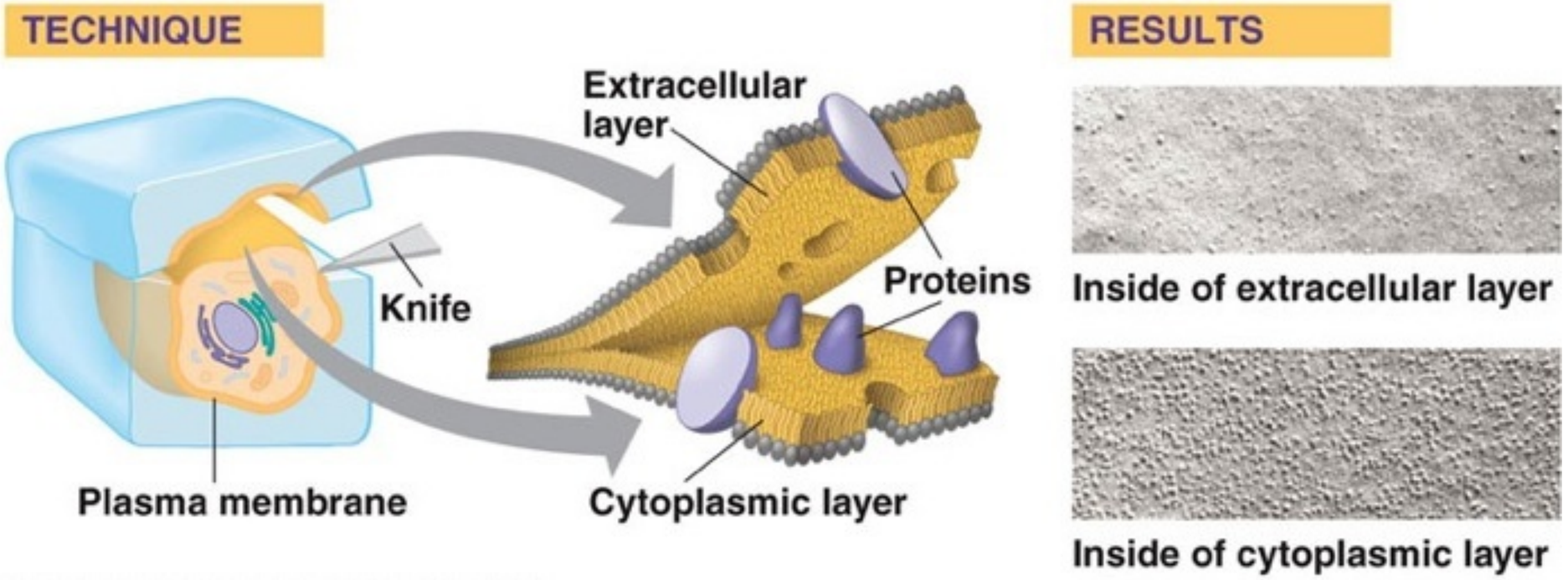


FIG. 6. Membranes frozen after a 3.0-sec exposure to an electric field of about 65 V/mm. Arrows indicate direction of migration of intramembrane particles towards the positive electrode. Convex (*Upper*), and concave and convex (*Lower*) fracture faces reveal migration of intramembrane particles toward the positive electrode. (*Upper*, ×55,000; *Lower*, ×62,000.)

Freeze fracture method (electron microscopy)



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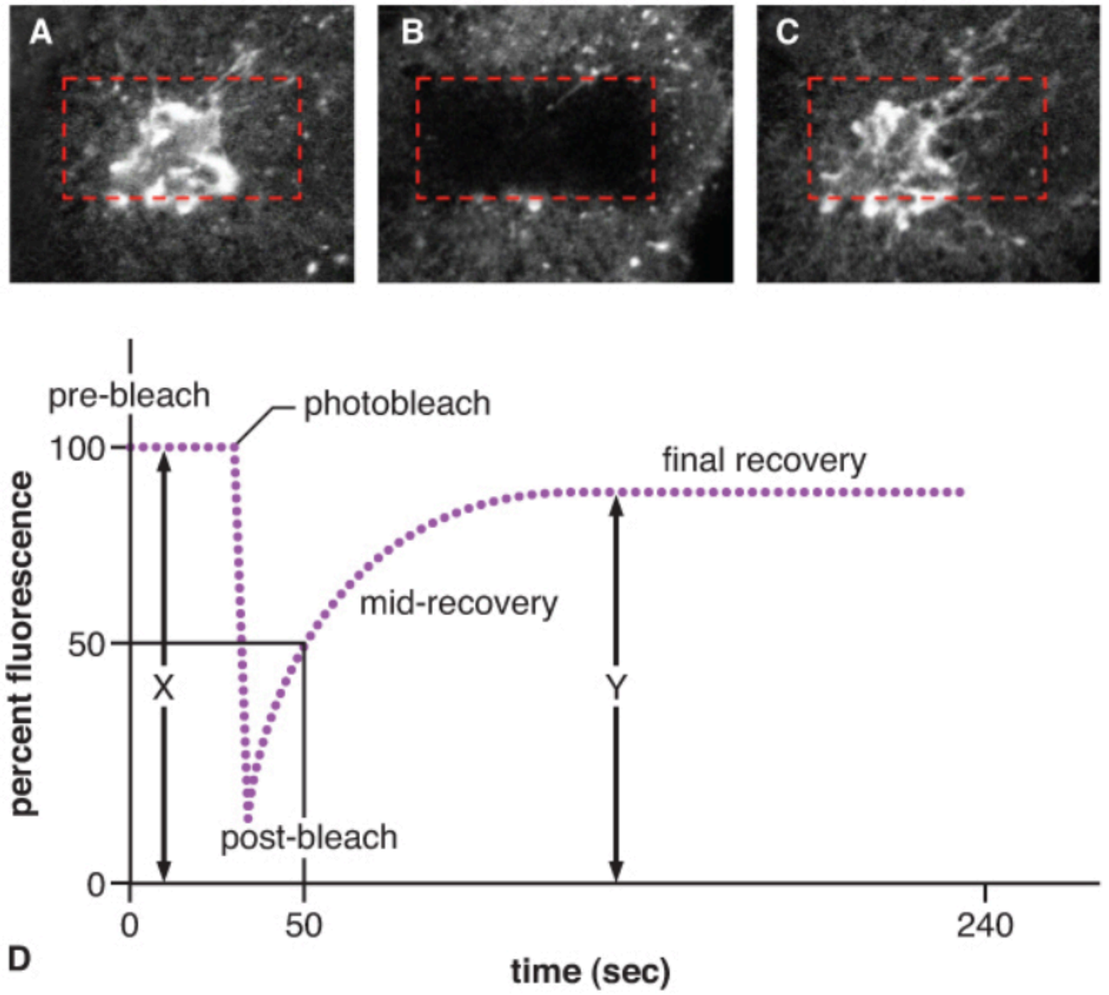


Figure 8.15 FRAP method. Cell immediately before **(A)** and after **(B)** photobleaching inside the boxed area. **C,** After about 2 minutes, the bleached area has recovered its glowing due to mobile proteins. **D,** Diagram of FRAP data showing key steps. A FRAP movie of the experiment is available online. A-C. Modified from a movie published as part of Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J. 1997. ER-to-Golgi transport visualized in living cells. Nature 389: 81-85. D. Common Knowledge

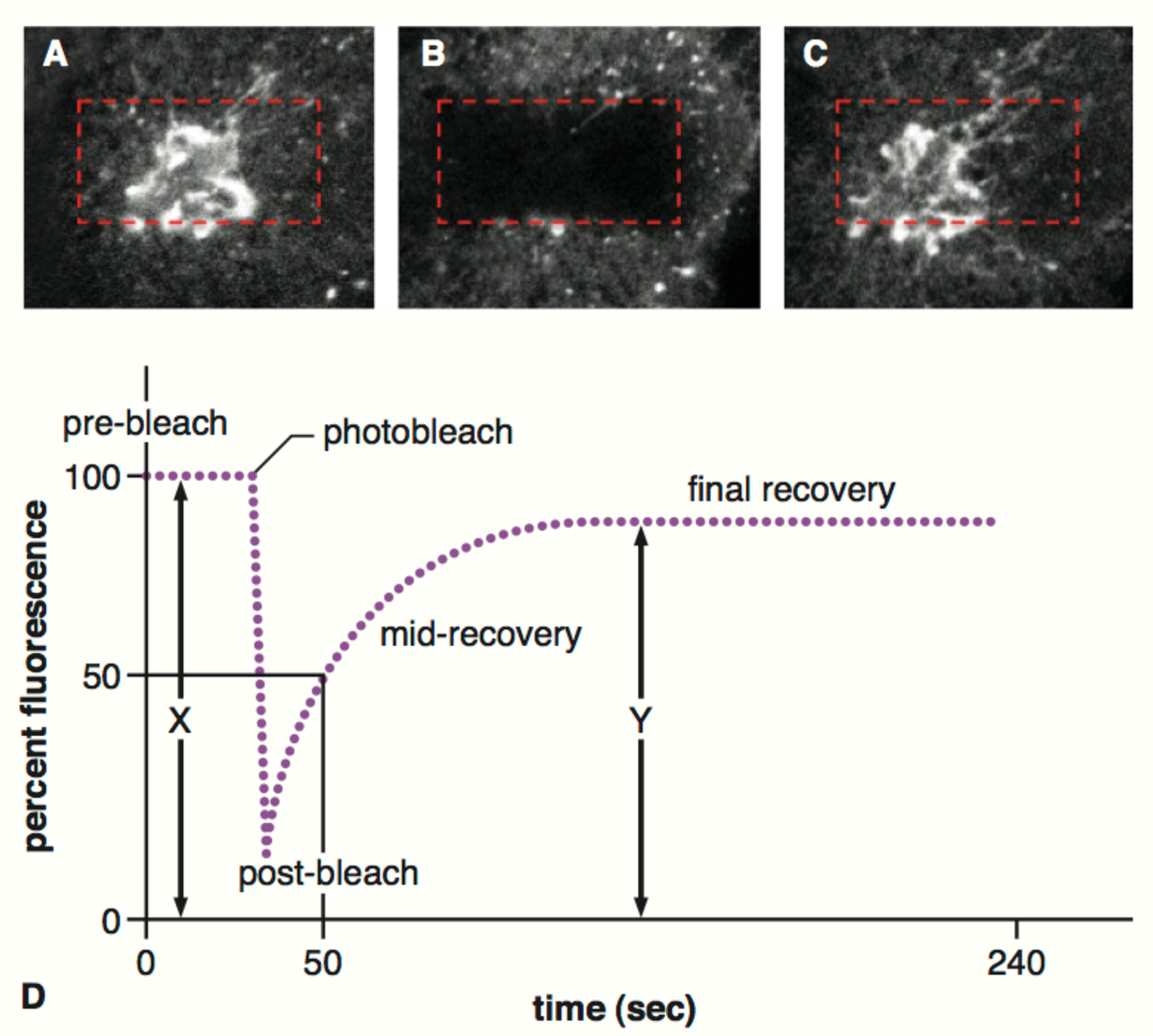


Fig. 8.15

A-C modified from Presley et al., 1997; B: original art

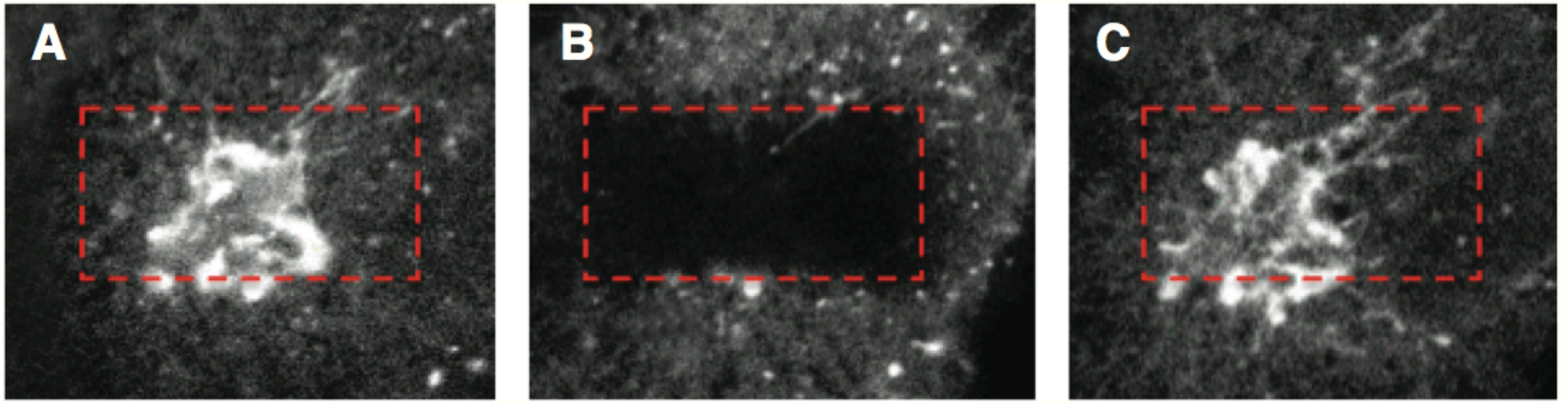
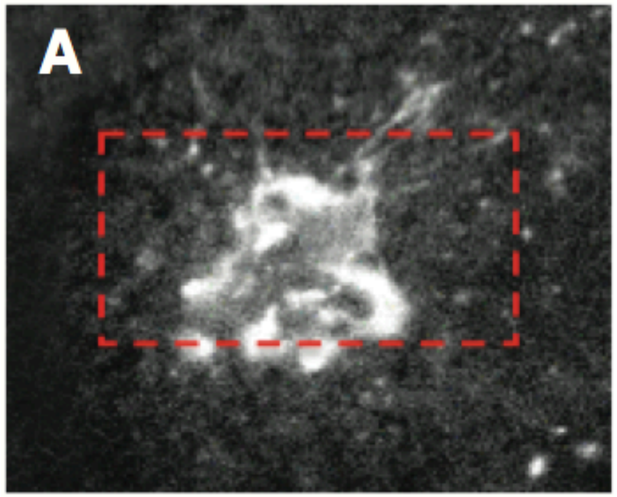


Fig. 8.15

A-C modified from Presley et al., 1997; B: original art

FRAP Measures Protein Movement



visualize proteins
targeted for photobleaching

Fig. 8.15

A-C modified from Presley et al., 1997; B: original art

FRAP Measures Protein Movement

photobleach proteins

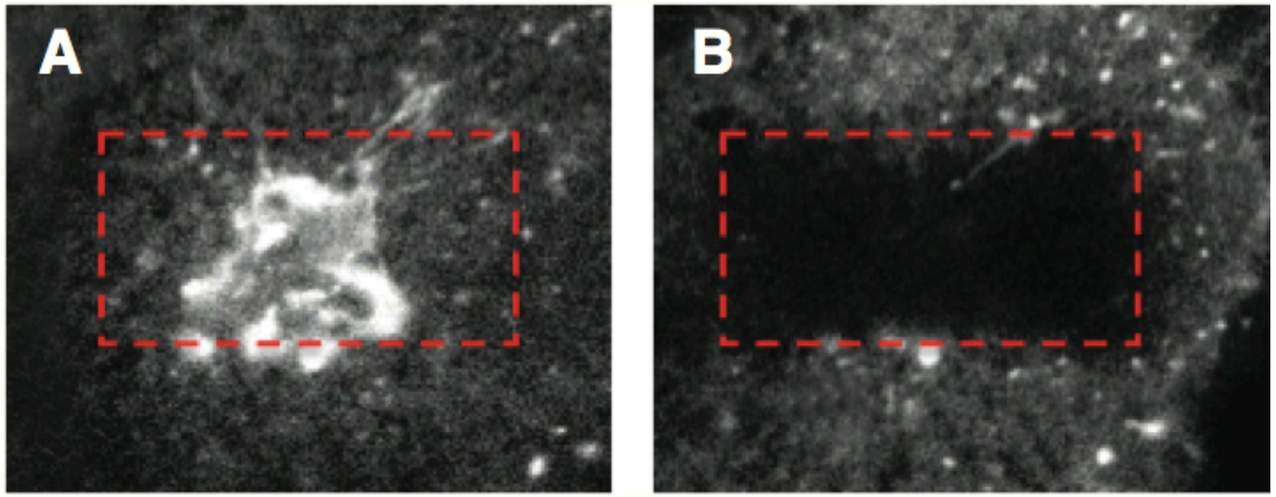
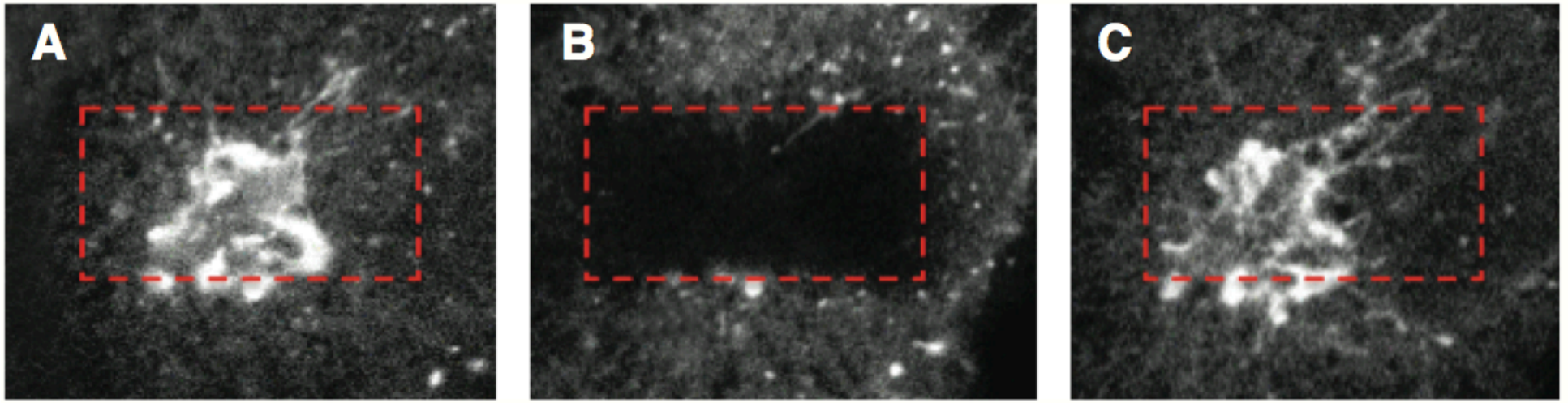


Fig. 8.15

A-C modified from Presley et al., 1997; B: original art

FRAP Measures Protein Movement



watch unbleached proteins move in

Fig. 8.15

A-C modified from Presley et al., 1997; B: original art

ER-to-Golgi transport visualized in living cells

John F. Presley, Nelson B. Cole, Trina A. Schroer*, Koret Hirschberg, Kristien J. M. Zaal & Jennifer Lippincott-Schwartz

Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, Building 18T, NICHD, NIH, Bethesda, Maryland 20892, USA

** Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218, USA*

Newly synthesized proteins tha (ER) are funnelled through the for transport to their differe approaches have elucidated t such transport[1–3] and have esta mediates[4–8]. New techniques fo have made it possible to follow transport intermediates in livi path and velocity *en route* to visualized ER-to-Golgi transp ts045 VSVG tagged with green Upon export from the ER, VS many differently shaped, rapi which translocated inwards t microtubules by using the motor complex of dynein/dyna rial from pre-Golgi structures o to the Golgi complex and they shapes. Together, our results in structures moving unidirectionally along microtubule tracks are

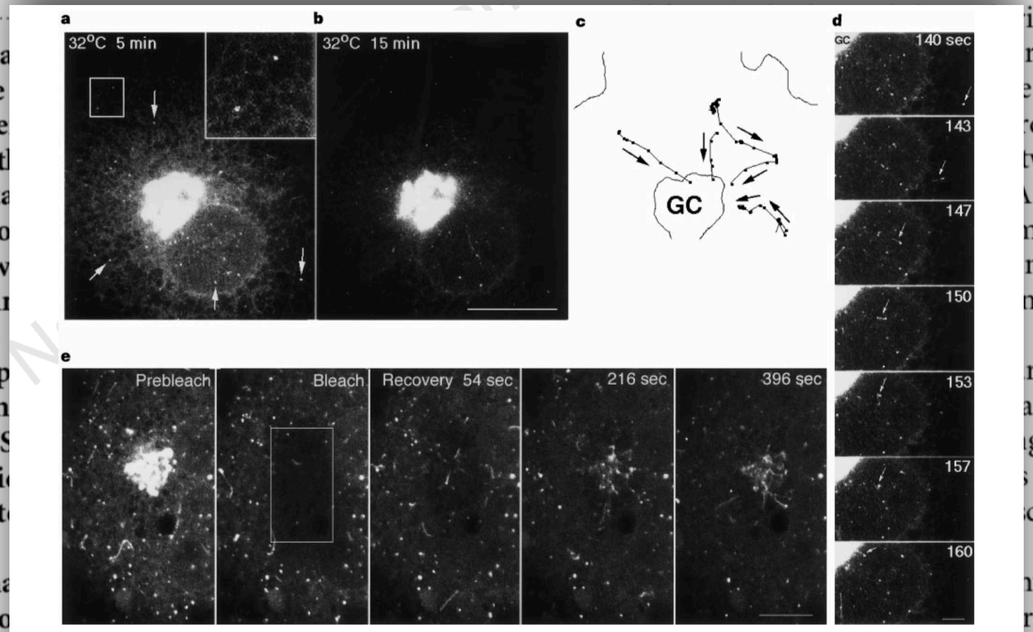


Figure 3 ER to Golgi transport of VSVG-GFP visualized upon shift from 40 °C to 32 °C or in cells whose Golgi area is photobleached. **a**–**d**, VSVG-GFP-expressing COS cells were incubated for 12 h at 40 °C and then shifted to 32 °C. **a**, Distribution of VSVG-GFP after 5 min at 32 °C. Arrows show examples of pre-Golgi intermediates. **b**, Distribution of VSVG-GFP after 15 min at 32 °C. **c**, Tracings of pre-Golgi intermediates moving towards the Golgi complex (GC). **d**, Image series showing shape change of a pre-Golgi intermediate as it translocated to the Golgi complex over the time interval shown. Scale bar, 4 μm. **e**, VSVG-GFP-expressing COS cells were incubated for 12 h at 40 °C, shifted to 15 °C for 3h and then warmed to 32 °C. Fluorescence associated with the Golgi

incubation for 3 h at 15 °C, co-localizing extensively with β-COP (Fig. 1a; 15 °C) and ERGIC53 (data not shown).

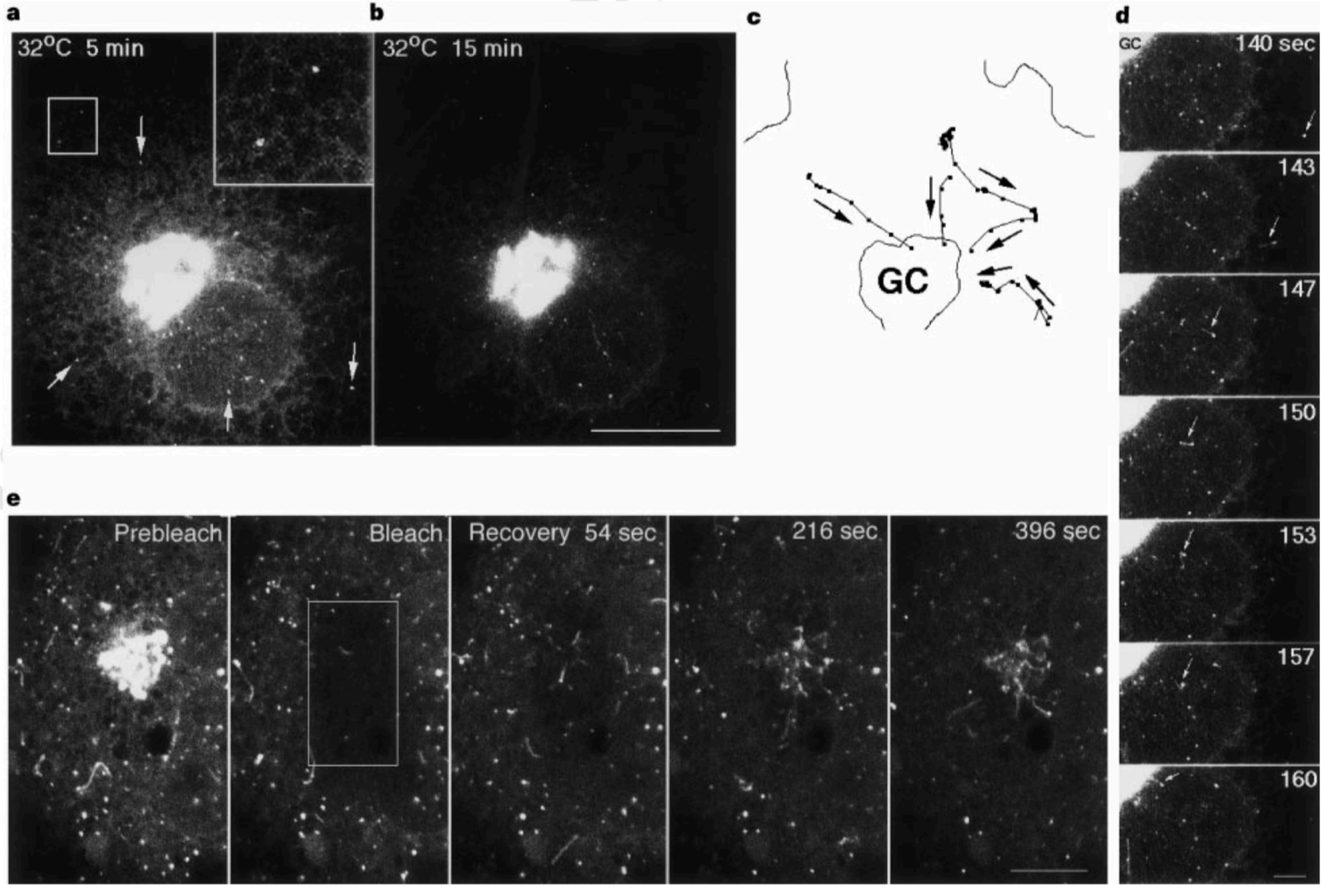
To examine how VSVG-GFP is transported from such intermediates into the Golgi region, cells expressing the chimaeric proteins at 15 °C were placed on a microscope stage warmed to 32 °C and fluorescent images were collected at 3.6-s intervals. As shown in Fig. 1b and c (also see Quicktime movie at http://dir.nichd.nih.gov/CBMB/pb1labob.html), peripheral pre-Golgi structures containing VSVG-GFP translocated rapidly as units into the centrosomal region where they merged into the large fluorescent area marking the Golgi complex. Figure 1b (centre) maps the movement of several of these structures over the course of 9 min upon warm-up from 15 °C to 32 °C. Such structures moved along straight or curvilinear paths towards the cell centre at speeds of up to 1.4 $\mu m\,s^{-1}$. Individual pre-Golgi elements were often greater than 1.5 μm in

ing translocation. Stain-
ring this period revealed
e VSVG-GFP-containing
rds to the Golgi complex
two pre-Golgi structures
A single long tubule was
notion of the pre-Golgi
motor on the tip of the
membranes towards the

riable times after warm-
ashion toward the Golgi
gle element containing
s plotted in Fig. 2a and b.
cence intensity as they
that VSVG-GFP mole-
nto small vesicle carriers,
re into the Golgi region.
visualized upon shifting
32 °C (Fig. 3a–d and
Quicktime movie). Within 1 to 3 min of the shift to lower

incubation for 3 h at 15 °C, co-localizing extensively with β-COP (Fig. 1a; 15 °C) and ERGIC53 (data not shown).

To examine how VSVG-GFP is transported from such intermedi-



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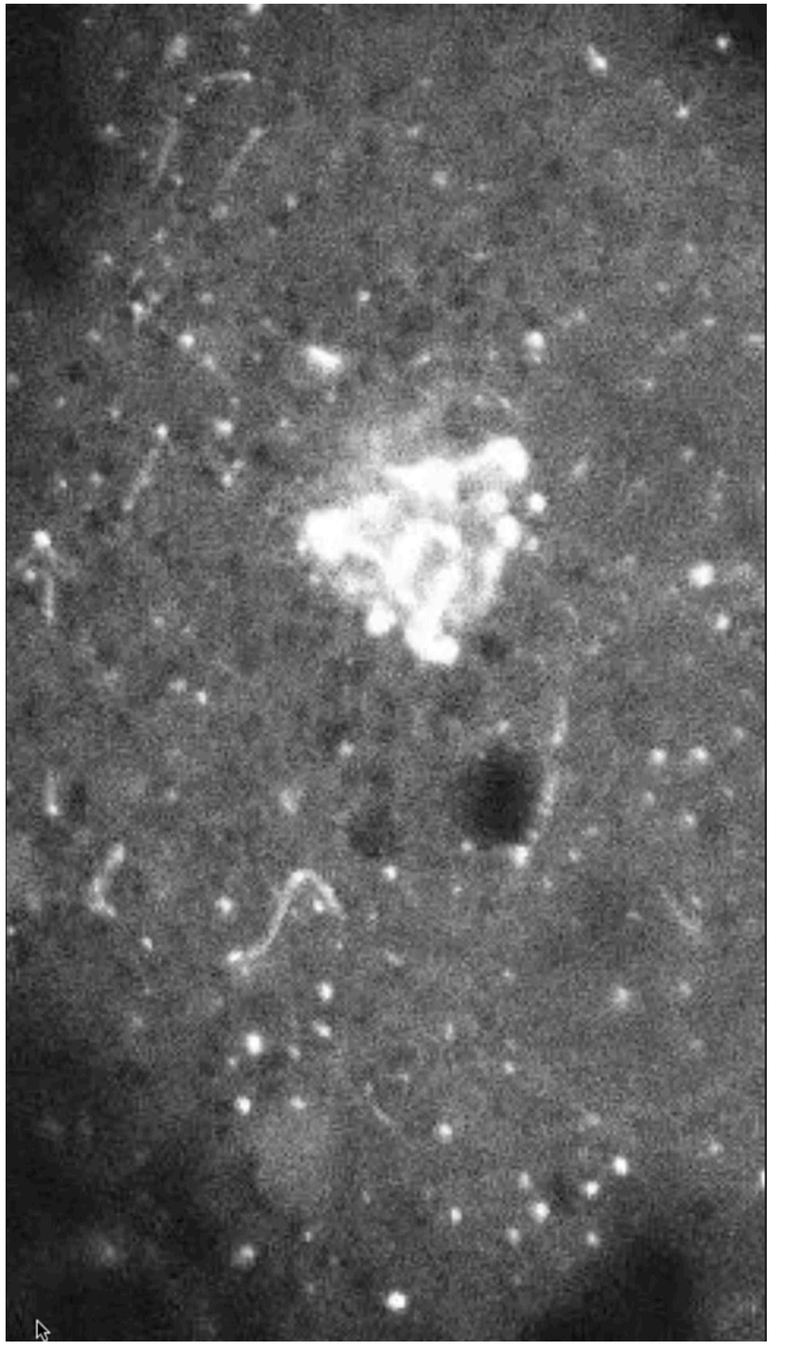
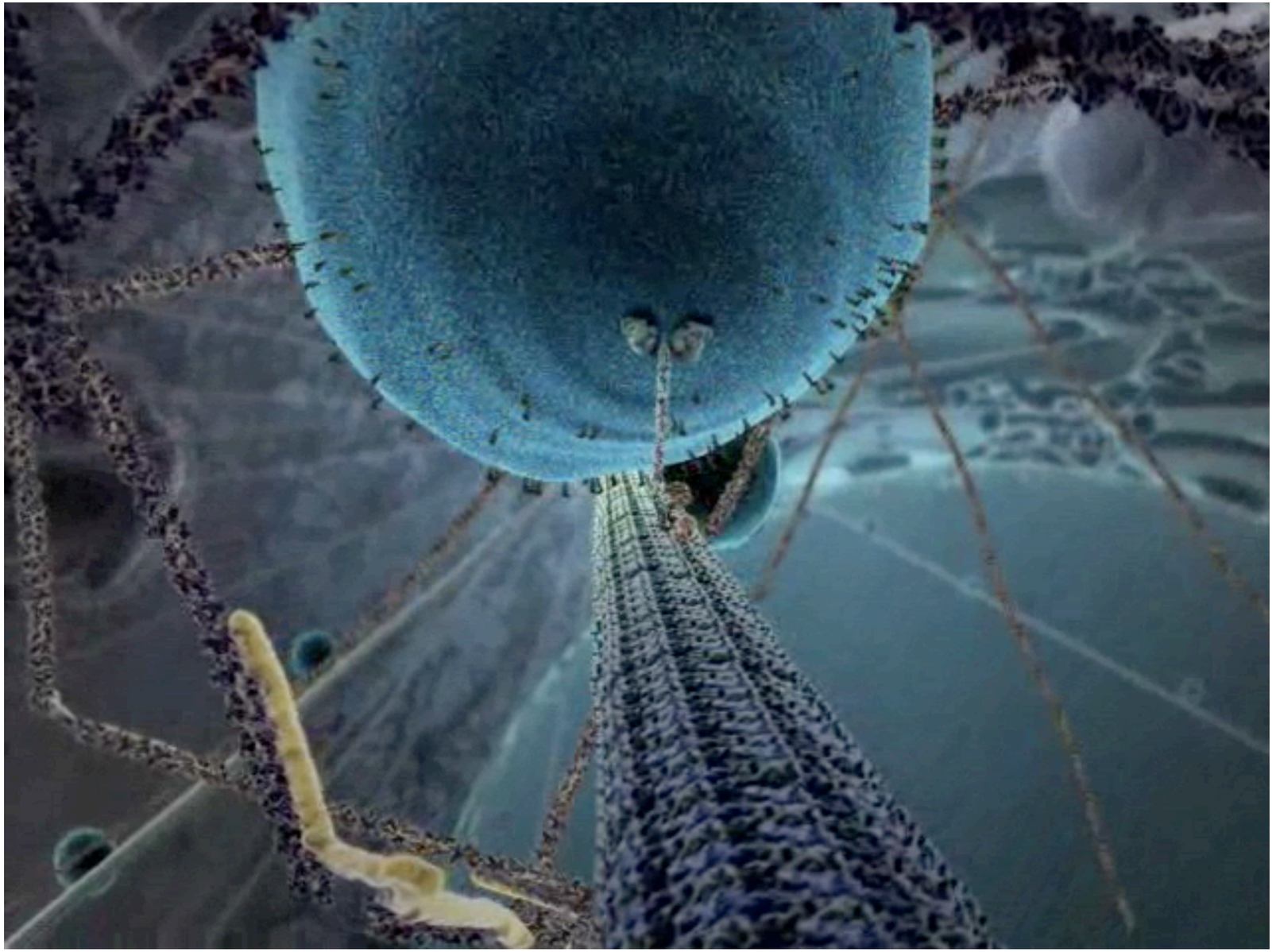


WHAT YOU WANT TO DO NEXT?

DID CLICKER QUESTIONS

WHAT YOU WANT TO DO NEXT?



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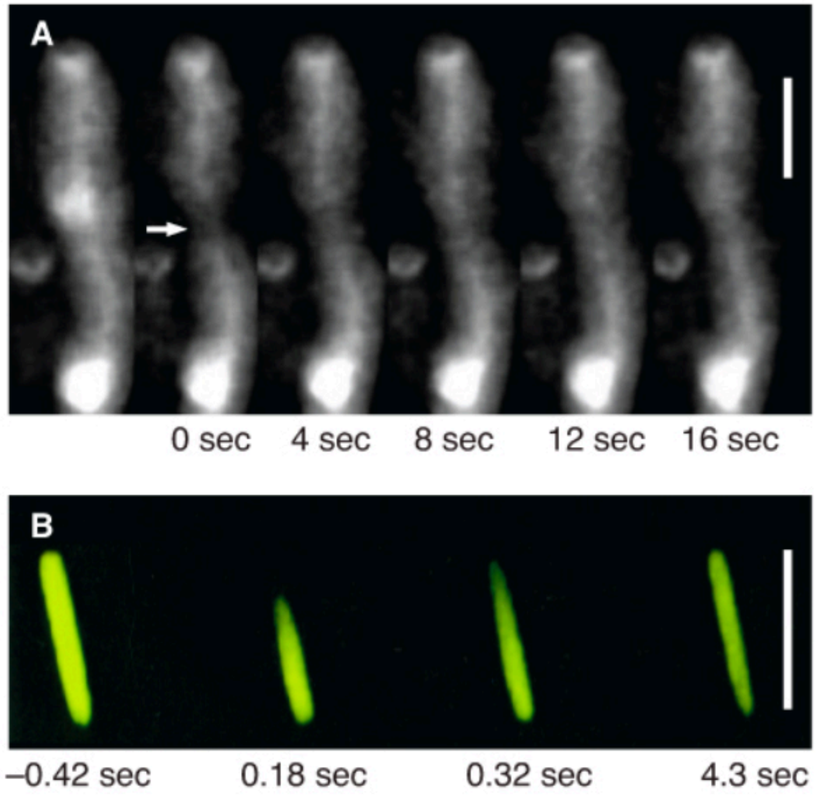

Figure 8.16 A, FRAP experiment with GFP fused with a normal plasma membrane protein; the arrow marks the area that was photobleached. The times are the seconds after bleaching. Bar = 5 µm. **B,** Imaged *E. coli* with GFP in the cytoplasm of the cell at the indicated times in seconds starting 0.42 seconds before photobleaching. Bar = 4 µm. Panel A from Mullineaux *et al.*, 2006, their figure 1. Mullineaux, Conrad W., Anja Nenninger, Nicola Ray, and Colin Robinson. 2006. Diffusion of Green Fluorescent Protein in Three Cell Environments in *Escherichia coli.* Journal of Bacteriology. Vol. 188(10): 3442–3448. B. From Elowitz *et al.*, 1999, modified from their figure 1. Elowitz, Michael B., Michael G. Surette, Pierre-Etienne Wolf, Jeffry B. Stock, and Stanislas Leibler. 1999. Protein Mobility in the Cytoplasm of *Escherichia coli.* Journal of Bacteriology. Vol. 181(1):197–203.

JOURNAL OF BACTERIOLOGY, Jan. 1999, p. 197–203 Vol. 181, No. 1
0021-9193/99/$04.00+0

Protein Mobility in the Cytoplasm of *Escherichia coli*

MICHAEL B. ELOWITZ,[1,2]* MICHAEL G. SURETTE,[2]† PIERRE-ETIENNE WOLF,[1,2]‡
JEFFRY

Departments of Physics[1] *and M*

Rece

**The rate of protein diffusion in ba
the rates of many biochemical reac
apparent diffusion coefficient of gre
measurements were made in two w
red-emitting fluorescent state of GF
Biol. 7:809–812, 1997). The apparen
2.5 μm²/s. A 72-kDa fusion protein
domain moves more slowly, with D_a
least two factors: first, D_a is reduced
to GFP of a small tag consisting of s
cytoplasmic viscosity cannot explain
the understanding of intracellular b**

Response times and reaction rates in *Esch*
depend on the movement of proteins from
another in the cell. These proteins may ha
signaling functions, or they may act as enzyn
for cellular reactions. How do such molecules

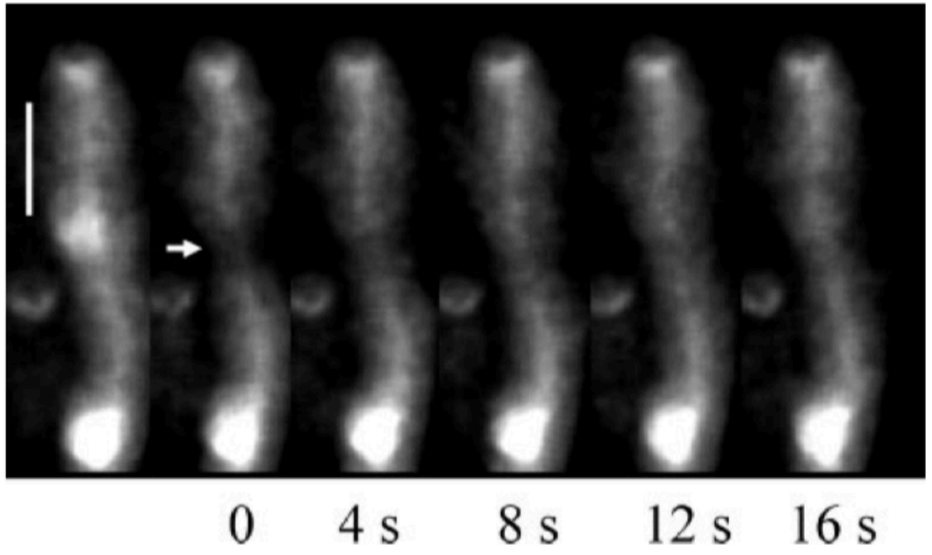

FIG. 1. FRAP image sequence for an elongated *E. coli* cell expressing TatA-GFP localized in the plasma membrane. The far left image is prior to the bleach. The next image is after bleach, and subsequent time in seconds is indicated. The center of the bleach is indicated by the arrow, whose thickness corresponds to the approximate beam width. Scale bar, 5 microns.

JOURNAL OF BACTERIOLOGY, May 2006, p. 3442–3448 Vol. 188, No. 10
0021-9193/06/$08.00+0 doi:10.1128/JB.188.10.3442–3448.2006

Diffusion of Green Fluorescent Protein in Three Cell Environments in *Escherichia Coli*

Conrad W. Mullineaux,[1]* Anja Nenninger,[2] Nicola Ray,[2] and Colin Robinson[2]

School of Biological and Chen... United Kingdom,[1] and Depar... 4NS, ...dom[2]

Surprisingly little is kn... rates at which proteins an... a physical system. There h... using fluorescence recover... is available about diffusion... of significant respects. We... cells of *Escherichia coli* el... cytoplasm, exported into t... integral plasma membran... a diffusion coefficient com... showed a very low rate of p... coefficient only slightly s... compartments are relative...

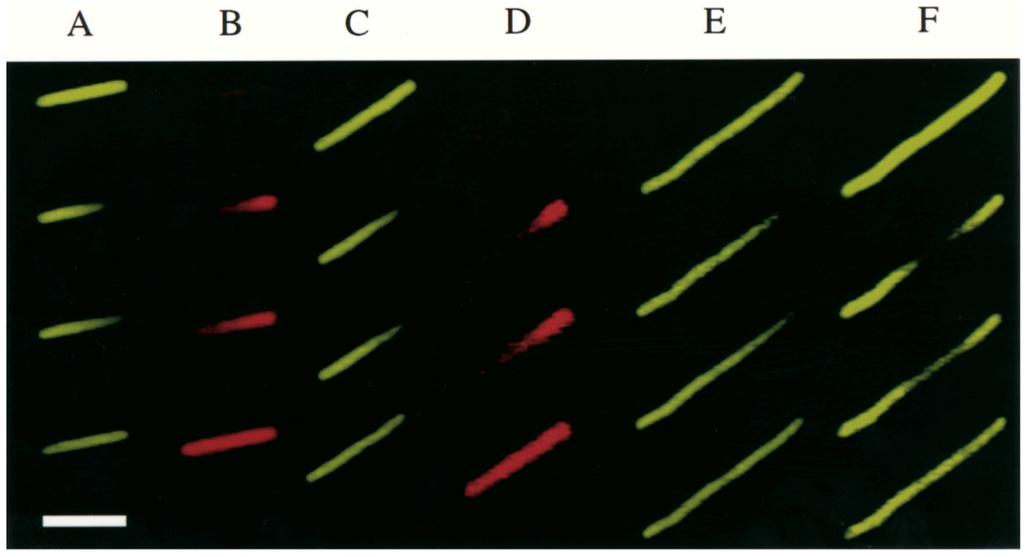

FIG. 1. Snapshots from photobleaching and photoactivation experiments. In each column the first row shows the cell before the laser pulse. The next three images show the cellular fluorescence distribution at subsequent times after the laser pulse. Columns A, C, E, and F show photobleaching (GFP filter set, false color green). Columns B and D show photoactivation (rhodamine filter set, false color red). Columns A to D show two different DH5α cells expressing GFP (A and B show cell 1; C and D show cell 2). Columns E and F show a cephalexin-treated DH5α cell, expressing GFP, being bleached first at the pole (E) and then at the center (F). Time points are as follows (t = 0 is set arbitrarily as the end of the laser pulse). (A) −0.42, 0.05, 0.18, 0.32, and 4.3 s. (B) −0.08, 0.08, 0.35, 0.62, and 4.7 s. (C) −0.5, 0.03, 0.10, 0.23, and 0.83 s. (D) −0.1, 0.03, 0.23, 0.63, and 1.7 s. (E) −0.57, 0.03, 0.43, 0.77, and 2.8 s. (F) −0.57, 0.03, 0.20, 0.37, and 1.8 s. Bar = 4 μm.

The diffusion of cell components is ... e of cephalexin, which inhibits cell division, causing the production of greatly elongated cells (4, 8, 19). Typically, a line is bleached across the short axis of the cell, and diffusion is observed in one dimension along the long axis of the cell (8). Such all living cells. Diffusion may be particularly important in prokaryotes, where systems of active transport appear to be much less developed than those in eukaryotes. We do not know enough about intracellular environments to be able to predict

Table 8.3

Table 8.3 Mobility of proteins in and on *E. coli* ± 1 standard deviation.

protein	location	diffusion rate	fold slower
GFP	water	87.0 ± 2.0	n.a.
GFP	cytoplasm *E. coli*	8.0 ± 2.3	~10 X
GFP over produced	cytoplasm *E. coli*	3.6 ± 0.7	~24 X
GFP + protein with sugar	cytoplasm *E. coli*	2.5 ± 0.6	~35 X
GFP	periplasm *E. coli*	2.6 ± 1.2	~33 X
GFP + membrane protein	membrane *E. coli*	0.13 ± 0.03	~669 X

data from Mullineaux *et al.*, 2006.