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followed by the automated cutting of membrane strips. Alternatively, they could be applied as lines, followed by cutting of the membrane in thin strips perpendicular to the application lines. Glass slides could be used instead of nylon. Photolithographic methods have been developed to generate two different oligonucleotides in one spot on silanated glass (5). However, the instability of the attachment of oligonucleotides attached to coatings on glass slides to the high temperatures of PCR (8) may hamper the application of this method. Less expensive methods of covalent attachment of modified oligonucleotides to derivatized glass surfaces need to be explored to identify an attachment method compatible with PCR amplification conditions. In addition, the flatness of most microarray slides may interfere with the access of the polymerase to the oligonucleotides. Thus, a combination of the attachment method and glass, nylon, or other surface compatible with PCR is key for the automation of the ViSA card method to detect multiple viruses simultaneously and should lead to a marriage of microarray and PCR technologies for many diagnostic applications.

REFERENCES

1. Chizhikov, V., A. Rasooly, K. Chumakov, and D.D. Levy. 2001. Microarray analysis of microbial virulence factors. *Appl. Environ. Microbiol.* 67:3258-3263.
2. Corne, J.M., S. Green, G. Sanderson, E.O. Caul, and S.L. Johnston. 1999. A multiplex RT-PCR for the detection of parainfluenza viruses 1-3 in clinical samples. *J. Virol. Methods* 82:9-18.
3. d'Offay, J.M., R.E. Mock, and R.W. Fulton. 1993. Isolation and characterization of encephalitic bovine herpesvirus type 1 isolates from cattle in North America. *Am. J. Vet. Res.* 54:534-539.
4. Ely, R.W., J.M. d'Offay, A.H. Ruefer, and C.Y. Cash. 1996. Bovine herpesviral encephalitis: a retrospective study on archived formalin-fixed, paraffin-embedded brain tissue. *J. Vet. Diagn. Invest.* 8:487-492.
5. Gentalen, E. and M. Chee. 1999. A novel method for determining linkage between DNA sequences: hybridization to paired probe arrays. *Nucleic Acids Res.* 27:1485-1491.
6. Gusmao, L., A. Gonzalez-Neira, C. Pestoni, M. Brion, M.V. Lareu, and A. Carracedo. 1999. Robustness of the Y STRs DYS19, DYS389 I and II, DYS390 and DYS393: optimization of a PCR pentaplex. *Forensic Sci. Int.* 106:163-172.

7. Lockley, A.K., C.G. Jones, J.S. Bruce, S.J. Franklin, and R.G. Bardsley. 1997. Colorimetric detection of immobilised PCR products generated on a solid support. *Nucleic Acids Res.* 25:1313-1314.
8. Rehman, F.N., M. Audeh, E.S. Abrams, P.W. Hammond, M. Kenney, and T.C. Boles. 1999. Immobilization of acrylamide-modified oligonucleotides by co-polymerization. *Nucleic Acids Res.* 27:649-655.
9. Sheppard, M. and J.T. May. 1989. Location and characterization of the bovine herpesvirus type 2 thymidine kinase gene. *J. Gen. Virol.* 70:3067-3071.
10. Sullivan, D.G. and R.K. Akkina. 1995. A nested polymerase chain reaction assay to differentiate pestiviruses. *Virus Res.* 38:231-239.
11. Thompson, M. and L.M. Furtado. 1999. High density oligonucleotide and DNA probe arrays for the analysis of target DNA. *Analyst* 124:1133-1136.

The authors wish to thank Dr. Robert Fulton and Lurinda Burge for preparation and provision of various BVDV samples. This project was supported by the Oklahoma Agricultural Experiment Station, whose Director has approved the work for publication. Address correspondence to Dr. Ulrich Melcher, Department of Biochemistry and Molecular Biology, 246 NRC, Oklahoma State University, Stillwater, OK 74078, USA. e-mail: u-melcher-4@alumni.uchicago.edu

Received 17 April 2001; accepted 21 August 2001.

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Live-Cell Nucleocytoplasmic Protein Shuttle Assay Utilizing Laser Confocal Microscopy and FRAP

BioTechniques 32:80-87 (January 2002)

ABSTRACT

In eukaryotes, protein trafficking to and from the nucleus, or shuttling, has been demonstrated to be an important function for proteins that have vital roles in one or both subcellular compartments. Current techniques of detecting protein nuclear shuttling are extremely labor intensive and only statically visualize evidence of shuttling. Fluorescence recovery after photobleaching (FRAP), or fluorescence microphotolysis, has proven to be an effective method of analyzing protein dynamics in live cells, especially when coupled to GFP technology. Here, we describe a relatively simple in vivo protein nuclear shuttling assay that utilizes red fluorescent and green fluorescent protein fusions as substrates for FRAP using a laser confocal microscope. This technique is less time consuming than established shuttle assays, is internally controlled, and visualizes nucleocytoplasmic shuttling in living cells of the same species and cell type. This technique can be potentially used to detect the ability of any nuclear protein to shuttle from the nucleus to any other subcellular compartment for any eukaryotic species in which GFP or dsRed1 fusion protein can be expressed.

INTRODUCTION

During the normal eukaryotic cell life cycle, every protein in the cell greater than approximately 25 kDa in size must be regulated to either be excluded from or included to the nucleus (2). Several unique protein nuclear localization signals (NLSs) have been described as modular polypeptide sequences that can modulate the nuclear import of any heterologous fused protein (15). There also exist nuclear export signals (NESs) that can signal the export of a protein from the nucleus to the cytoplasm (2,15). Some proteins have vital roles in both subcellular compartments

or shuttle from the nucleus to the cytoplasm to carry out their function (14,15).

Nuclear transport factors themselves, such as the importin family of proteins (14), shuttle to and from the nucleus (14,15). The shuttle capability of nuclear transport factors is essential for these factors to be able to recycle to the nuclear or cytoplasmic compartment for another round of cargo import or export (2,14,15).

One established technique for the study of protein nucleocytoplasmic shuttling is by protein micro-injection (4,11). Micro-injection of purified and fluorescently labeled recombinant protein into one nucleus of a multi-nuclear cell (polykaryon) can identify if the protein of interest is capable of shuttling (11). Although effective, this technique is technically difficult and time consuming and requires a large amount of soluble, purified protein.

The most established method of assaying a nuclear protein's ability to shuttle is the heterokaryon assay (4,6). Briefly, this assay involves culturing transfected human and untransfected mouse cultured cells. Protein shuttling is observed from the transfected human nucleus to the mouse nucleus of a heterokaryon. The shortcomings of this technique are the requirement of a very specific antibody to the test protein and the requirement that the mouse nuclear import pathway utilized by the test protein be very highly conserved between human and mouse species. This cross-species barrier could potentially miss the shuttle ability of a human protein to the nucleus of another species. The technique requires over 30 h of manipulation 24 h after transfection. It can also be very difficult to interpret a negative result, as there is no way to ensure that a true heterokaryon was formed.

GFP (12) and red fluorescent protein (dsRed1) (1) have developed into common reagents for the visualization of fusion proteins expressed in living cells. In addition to relatively bright fluorescence in living cells, GFP and dsRed1 fusions can be effectively photobleached with appropriate lasers via a laser confocal microscope (7,13). Photobleaching occurs when a focused, high-energy laser light is applied to a fluorophore, resulting in the saturation of the fluorochrome and subsequent

permanent loss of fluorescence (7,8). Bleaching the GFP/dsRed1 fusions in one subcellular compartment of the cell and observing the recovery of fluorescence from molecules either actively transporting or diffusing from another unbleached part of the cell is referred to as fluorescence recovery after photobleaching (FRAP) or fluorescence microphotolysis (5,7,8,13).

Our goal was to develop a simple, fast, real-time, live-cell method to test the ability of a protein with nuclear localization to shuttle. The method described here involves transiently transfected mammalian cells expressing GFP/dsRed1 fluorescent protein fusions that are treated briefly with polyethylene glycol (PEG) (Sigma, St. Louis, MO, USA) to allow cell fusion. Upon identi-

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fication of a bikaryon, all of the cell area except for one of the two nuclei is photobleached. Fluorescence recovery into the bleached nucleus indicates that the test protein has transport signals to shuttle from one nucleus to the other. This method is faster than previously described methods, uses only one cell type, visualizes living cells, is internally controlled, and recovery is observed in real time. This method could potentially be universally used to test for nuclear protein shuttling ability to and from the nucleus in any eukaryotic cell type that can express GFP/dsRed1 fusion proteins.

MATERIALS AND METHODS

Plasmid Expression Constructs

A positive control phnRNPA1-dsRed1 mammalian expression plasmid was constructed by PCR amplification (DeepVent™ DNA polymerase; New England Biolabs, Beverly, MA, USA) of mouse hnRNPA1 cDNA from a mouse heart cDNA library (generous gift of P. Whyte, McMaster University) with synthetic oligonucleotides introducing unique restriction sites. Following restriction endonuclease digestion, the PCR product was ligated into digested pdsRed-N1 vector (BD Biosciences Clontech, Palo Alto, CA, USA).

Negative control plasmid pHM840 was a generous gift of Thomas Staminger (10).

Test protein eGFP-importin α construct peGFP-Imp α , was constructed by PCR amplifying human importin α Rch1p cDNA from plasmid pGST-Imp α (11) with synthetic oligonucleotides introducing restriction endonuclease sites. The PCR product was digested with restriction endonucleases and ligated into peGFP-C3 vector (BD Biosciences Clontech).

All plasmid DNAs were prepared by cesium chloride equilibrium density gradient centrifugation. All DNA-modifying and restriction enzymes (*EcoRI* and *SacII*) used were purchased from New England Biolabs.

Construction of Coverslip-Bottomed Cell Culture Dishes

Standard 35 × 10 mm cell culture

dishes (Corning, Corning, NY, USA) were inverted, and a 15-mm hole was made on the bottom of the culture dish using a side-cutting drill (Dremel Products, Racine, WI, USA). The circumference of the hole was then continuously beaded with all-purpose silicone seal (GE Silicones, Waterford, NY, USA). A standard no. 1 thickness 22-mm square coverslip (VWR Scientific, Media, PA, USA) was then carefully pressed onto the sealant. Culture dishes were then allowed to dry for at least an hour, then sterilized by immersion into 70% ethanol, and allowed to air dry in a tissue culture hood under UV light just before use.

Transfected Cell Lines and Growth Conditions

Human epithelial carcinoma (HeLa) adherent cell line was used (ATCC, Manassas, VA, USA). Cells were grown in 10% FCS (Sigma) in DMEM (Sigma) supplemented with antibiotics and antimycotics (Invitrogen, Carlsbad, CA, USA) at 37°C under 5% CO₂. HeLa Cells were seeded at a density of 12.5×10^3 cells and allowed to incubate to 75% confluency (approximately 20 h). Seeded cells were then transfected by the calcium phosphate method (3) using 2 μ g phnRNPA1-RFP with 2 μ g peGFP test protein plasmid and incubated for a further 24–48 h.

Preparation of Cell Fusions

To generate bikaryon cells, transfected HeLa cells were treated with 50% PEG in PBS for 6 min, following aspiration of the growth medium. Cells were then washed four times with PBS and placed in 2 mL fresh growth medium supplemented with 50 μ g/mL cycloheximide (Calbiochem-Novabiochem, San Diego, CA, USA). Just before microscopy, the 2 mL cell culture media were further supplemented with 200 μ L 10× HEPES buffer (250 mM HEPES, pH 7.4, 1.6 M NaCl). All subsequent steps took place at 25°C.

Laser Confocal Microscopy and FRAP

All confocal microscopy was done on a Zeiss LSM510 inverted laser con-

focal microscope (Carl Zeiss, Thornwood, NY, USA) equipped with argon and helium-neon lasers. Live bikaryon cells were identified that exhibited strong nuclear fluorescence in both red and green channels and that contained only two nuclei in a shared cytoplasm (10–20 bikaryons/dish). One identified bikaryon cell was then scanned confocally under low laser power (12.5%) at 1.0 Airy units.

The fluorescent bikaryon was then photobleached in all the visible area but one nucleus for 50 iterations at maximum laser power (100%) by both the argon and helium-neon lasers simultaneously. Cells were then immediately scanned at low laser power again post-bleaching at settings equal to the pre-bleach scan and allowed to sit for 30 min before the second scan.

RESULTS AND DISCUSSION

Our goal was to develop a method to assay the shuttling of a nuclear protein in live cells. To properly initiate these experiments, we found it necessary to find a convenient way to visualize live cells by fluorescence microscopy directly in the culture dish. Our solution was to construct custom coverslip-bottomed cell culture dishes, as described in Figure 1A. These coverslip-bottomed culture dishes proved extremely practical in all manipulation steps of the assay, economical (at a fraction of the cost of commercially available alternatives), and simple to construct. The use of this culture dish allowed us to seed, transfect, fuse, and conduct the FRAP experiment without having to trypsinize, resuspend, or otherwise disturb the cells.

One aspect of the mouse-human heterokaryon assay that we needed to address was the lack of any internal control for shuttling activity. We decided to use a positive control shuttle fusion protein, hnRNPA1 (6,9), fused to the dsRed1 (1) with exclusive emission and excitation maxima from enhanced GFP (eGFP) (12) (Figure 2, C and F). We also chose this fluorescent protein because of its ability to be effectively photobleached by a 543-nm helium-neon laser in a typical laser confocal microscope (Figure 2, D and E).

The theory was to co-transfect our

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test protein as a GFP fusion together with hnRNPA1-dsRed1 and observe fluorescence in the red, green, and differential interference contrast (DIC)

channels simultaneously with a laser confocal microscope after fusing transfected cells together with PEG (Figure 1C). Once a bikaryon is detected, a cell

containing two red and green fluorescing nuclei in a shared cytoplasm, all of the cell area but one of the nuclei is photobleached by both 488-nm argon and 543-nm helium-neon lasers at maximum power (Figure 1D). We then scan the cells at low laser power periodically post-bleaching to attempt to observe FRAP. The positive internal control was fluorescence recovery in the bleached nucleus of our hnRNPA1-dsRed1 protein at all times if the cell is a true bikaryon and not just two cells overlaying each other (Figure 1B). In the green channel, we expect to see fluorescence recovery in the bleached nucleus only if the protein has shuttle ability, the ability to export from the unbleached nucleus (donor) to the cytoplasm and import to the bleached nucleus (acceptor). FRAP is carried out in the presence of cycloheximide, an inhibitor of translation, to prevent artifactual recovery as a result of new protein synthesis. The area photobleached includes the entire cell area except for the donor nucleus (Figure 1C). This is to ensure that the fluorescence recovery detected is in fact coming from the donor nucleus and not from any cytoplasmic protein.

To perform laser photobleaching, we used a Zeiss LSM510 laser confocal microscope. One unique feature of this laser confocal microscope is its ability to precisely define odd shapes for a scan area (Figure 1C). A conventional laser confocal microscope could mimic the same bleach pattern with overlapping squares.

Our first experiments with this assay involved a known shuttle protein, hnRNPA1 (6), and a protein that contains an NLS only and therefore should not shuttle (Figure 2). This test protein is a 130-kDa triple-fusion protein of eGFP, the simian virus 40 large T antigen NLS and *E. coli* β -galactosidase (10). HeLa cells were transfected in our custom culture dishes and fused with PEG. In the constant presence of cycloheximide, the culture dish was examined for the presence of bikaryon cells in which both nuclei are fluorescing in the red and green channels (Figure 2G). On any given experiment, 10–20 bikaryons can be detected. Once a bikaryon was detected, we then selected a scan area that allowed the entire area within the cell to

Figure 1. Schematic of the nucleocytoplasmic FRAP shuttle assay. (A) Construction of coverslip-bottomed culture dishes. Step I: a 15-mm circle is marked on the bottom of a standard 35 × 10 mm cell culture dish. Step II: circular hole is cut from the plastic. Step III: a continuous bead of silicone adhesive is applied to the circumference of the hole. Step IV: a standard no. 1 thickness 22-mm coverslip is pressed firmly onto the adhesive. (B) Schematic time course of the assay. Merged red and green fluorescence is indicated with the gray-scaled nuclei; bleaching is indicated by the white nuclei. (C) Possible outcomes of the assay: (i) fluorescence recovery in red and green detection channels in the acceptor nucleus indicating that the test protein (green) has exported from the donor nucleus and imported into the acceptor (bleached) nucleus; (ii) fluorescence recovery in the red channel only, indicating that the cell is a fused bikaryon with its nuclear trafficking machinery intact, but the test protein does not shuttle; and (iii) no fluorescence recovery, indicating that the cells did not fuse or that the cells are otherwise not viable for nucleocytoplasmic transport. (D) The photo-bleach pattern. The bleach mask pattern over the bikaryon is indicated by the black lines around the indicated donor nucleus. The bleached area including the acceptor nucleus is indicated by the arrows.

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be bleached, with the exception of the donor nucleus, by argon and helium-neon lasers simultaneously (Figure 1C).

Initially, this photobleaching step required optimization. Empirically, our solution was to bleach with both lasers for 50 iterations (Figure 2, B, E, and H). This provided the minimum amount of energy required to bleach the cells without any light-induced toxicity over the time course of the experiment.

Recovery of hnRNPA1-dsRed1 fluorescence could be observed in the red channel in as little as 10 min, peaking at 30 min post-bleaching (Figure 2, F and I). No recovery was seen for eGFP-NLS- β -gal after 30 min (Figure 2, C and I), or after two hours, at which time the experiment was stopped (data not shown). For the negative result, the hnRNPA1-dsRed1 is a good internal control because it fluoresces at about half the intensity of eGFP (1). Since dsRed1 fluorescence is about half that of eGFP (1), and recovery has been observed to be half as sensitive than eGFP; therefore, the lack of eGFP recovery indicates a true negative result. Thus, the NLS-containing test protein did not shuttle, while the internal control hnRNPA1-dsRed1 fusion protein shuttled from the donor nucleus to the acceptor nucleus. The internal control hnRNPA1-dsRed1 confirmed that the cells were true bikaryons and their nucleocytoplasmic import/export pathways were intact.

With positive and negative controls for the assay functioning correctly, our next experiment was to see if another known nucleocytoplasmic shuttle protein, the import factor importin α (14) could function in the time parameters of this assay. Unlike hnRNPA1, importin α enters the nucleus via its interaction with the general nuclear import factor, importin β (karyopherin β , or yeast Kap95p) (14). Importin α and importin β are then exported back through the nuclear pore complex to the cytoplasm for another round of NLS-cargo import (11,14,15).

As in Figure 2, eGFP-importin α was co-transfected with hnRNPA1-dsRed1 (Figure 3). As with the previous experiment, dual-channel fluorescing bikaryons were identified and photobleached (Figure 3, G and H). Unlike the previous experiment, however, fluorescence recovery was seen in

both the red and green channels (Figure 3, C, F, and I), indicating that both importin α and hnRNPA1 fusions shuttled from one nucleus to the other of the bikaryon. We also observed from this experiment that a portion of the eGFP-importin α remained in the cytoplasm (Figure 3F). This cytoplasmic recovery is presumably due to the fact that importin α can recognize several NLS-containing target proteins in the cytoplasm. This may also explain why, in both the case of hnRNPA1-dsRed1 and eGFP-importin α , fluorescence equilibrium is never observed between the donor and acceptor nucleus (Figures 2F and 3F), as is the case with mouse-human heterokaryon assays (9).

We have demonstrated an effective technique to study the ability of a nuclear localized protein to shuttle from the nucleus to the cytoplasm. The total

time required for this assay is no more than 1 h following 24 h post-transfection, versus 30 or more hours for other techniques. This technique should be applicable to any nuclear protein that retains function as a GFP fusion in any cell type that can express GFP proteins and tolerate cell fusion. The obvious advantages of this technique are that bikaryons are formed within the same cell type, avoiding potential cross-species artifacts, and the internal control. Another advantage is that the cells are alive and recovery can be observed in real time, with relative qualitative differences readily observed between different nuclear shuttle proteins. One caveat to this assay, as with any assay that involves fusion proteins, is that the test GFP or dsRed1 fusion protein localization needs to be compared to the unfused protein to ensure that subcellu-

Figure 2. Positive nucleocytoplasmic shuttling of hnRNPA1-dsRed1 but not an eGFP-NLS-only-containing protein. Laser confocal microscope images of fluorescent and DIC channels of hnRNPA1-dsRed1 and eGFP-NLS- β -gal fluorescence in a bikaryon with the green and DIC channels merged (A, B, and C), red and DIC channels merged (D, E, and F), and both red and green and DIC channels merged, indicated as yellow (G, H, and I). Pre-, post-, and 30 min post-bleach are indicated for each of the channels. Fluorescence recovery of hnRNPA1-dsRed1 can be seen comparing the top (acceptor) nucleus in panels E and F or H and I. No fluorescence recovery could be seen for eGFP-NLS- β -gal protein when comparing the top acceptor nucleus post-bleaching in panels B and C or H and I. Scale bar is approximately 10 μ m.

Figure 3. Positive nucleocytoplasmic shuttling of hnRNP A1-dsRed1 and eGFP-importin α proteins. Laser confocal microscope images of merged fluorescent and DIC channels of hnRNP A1-dsRed1 and eGFP-importin α fluorescence in a bickaryon with the green and DIC channel merged (D, E, and F), red and DIC channel merged (A, B, and C), and both red and green channels merged with DIC, indicated as yellow (G, H, and I). Pre-, post-, and 30 min post-bleach. Fluorescence recovery of hnRNP A1-dsRed1 can be seen comparing the top (acceptor) nucleus in panels B and C or H and I. FRAP could be seen for eGFP-importin α protein when comparing the top acceptor nucleus post-bleaching in panels E and F or H and I. Some fluorescence recovery of eGFP-importin α , but not hnRNP A1-dsRed1, to the cytoplasm can be observed in panels E and F or H and I. Scale bar is approximately 10 μ m.

lar localization is not affected. Other possible concerns are that dsRed1 protein forms tetramers (1) and can aggregate under high over-expression conditions (1) that could potentially interfere with shuttling ability. To date, we have not observed this with several dsRed1 fusions or hnRNP A1. This assay can potentially allow the study of any nuclear protein, in any cell type, to determine if it has the ability to shuttle to and from the nucleus and provide further insight to its function in cell biology.

REFERENCES

1. Baird, G.S., D.A. Zacharias, and R.Y. Tsien. 2000. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad. Sci. USA* 97:11984-11989.
2. Gorlich, D. and U. Kutay. 1999. Transport

between the cell nucleus and the cytoplasm. *Ann. Rev. Cell Dev. Biol.* 15:607-660.

3. Graham, F.L. and A.J. van der Eb. 1975. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 2:456-467.
4. Johnson, C., D. Van Antwerp, and T.J. Hope. 2000. An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of I κ B α . *EMBO J.* 18:6682-6693.
5. Lippincott-Schwartz, J., E. Snapp, and A. Kenworthy. 2001. Studying protein dynamics in living cells. *Nat. Rev. Mol. Cell Biol.* 6:444-456.
6. Michael, W.M., M. Choi, and G. Dreyfuss. 1995. A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. *Cell* 83:415-422.
7. Peters, R. 1981. Translational diffusion in the plasma membrane of single cells as studied by fluorescence microphotolysis. *Cell. Biol. Int. Rep.* 5:733-760.
8. Peters, R. 1983. Nuclear envelope permeability measured by fluorescence microphotolysis of single liver cell nuclei. *J. Biol. Chem.*

258:11427-11429.

9. Pollard, V.W., W.M. Michael, S. Nakielnny, M.C. Siomi, F. Wang, and G. Dreyfuss. 1996. A novel receptor-mediated nuclear protein import pathway. *Cell* 86:985-994.
10. Sorg, G. and T. Stamminger. 1999. Mapping of nuclear localization signals by simultaneous fusion to green fluorescent protein and to β -galactosidase. *BioTechniques* 5:858-862.
11. Truant, R., R.A. Fridell, E.R. Benson, A. Herold, and B.R. Cullen. 1998. Nucleocytoplasmic shuttling by protein nuclear import factors. *Euro. J. Cell Biol.* 77:269-275.
12. Tsien, R.Y. 1998. The green fluorescent protein. *Annu. Rev. Biochem.* 167:509-544.
13. White, J. and E. Stelzer. 1999. Photobleaching GFP reveals protein dynamics inside live cells. *Trends Cell Biol.* 2:61-65.
14. Wozniak, R.W., M.P. Rout, and J.D. Aitchison. 1998. Karyopherins and kissing cousins. *Trends Cell Biol.* 5:184-188.
15. Yoneda, Y. 2000. Nucleocytoplasmic protein traffic and its significance to cell function. *Genes Cells* 10:777-787.

This laboratory is supported by operating grants from the Hereditary Disease Foundation USA and the Canadian Institutes of Health Research (CIHR). R.T. is supported by a CIHR New Scientist salary support award. Address correspondence to Dr. Ray Truant, McMaster University, 4H24B 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5. e-mail: truantr@mcmaster.ca

Received 26 June 2001; accepted 19 September 2001.

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