

Internuclear Exchange of an Inner Nuclear Membrane Protein (p55) in Heterokaryons: In Vivo Evidence for the Interaction of p55 with the Nuclear Lamina

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Abstract. The movement between nuclei of an integral protein of the inner nuclear membrane has been studied in rat/mouse and rat/hamster heterokaryons. This protein, p55, was found to equilibrate between nuclei over a period of ~ 6 h in the absence of new protein synthesis. When rat/mouse heterokaryons were constructed using an undifferentiated murine embryonal carcinoma (P19), which lacks lamins A and C, no accumulation of p55 in the mouse cell nucleus was observed. However, P19 nuclei could be rendered competent to accumulate p55 by transfecting the parent cells with human lamin A before cell fusion, supporting the notion that p55 may interact with the nuclear lamina. Since p55 does not appear to be able to dissociate from the nuclear membrane, it is concluded

that this exchange between nuclei does not occur in the aqueous phase and instead is probably membrane mediated. It is proposed that this protein may be free to move between the inner and outer nuclear membranes via the continuities at the nuclear pore complexes and that transfer between nuclei occurs via lateral diffusion through the peripheral ER, which appears to form a single continuous membrane system in these heterokaryons. One implication of these observations is that accumulation of at least some integral proteins in the inner nuclear membrane may be mediated by interactions with other nuclear components and may not require a single defined targeting sequence.

THE nuclear envelope has a complex organization consisting of several major structural elements. These are an outer and an inner membrane, nuclear pore complexes, and a nuclear lamina (Gerace and Burke, 1988; Newport and Forbes, 1987). The two membranes are in fact continuous, being periodically joined where they are penetrated by nuclear pore complexes, the latter forming the channels for the regulated exchange of macromolecules between the nucleus and the cytoplasm. In addition, the outer nuclear membrane is continuous with and forms part of the ER. The final major component of the nuclear envelope, the nuclear lamina, is a proteinaceous meshwork 10–20 nm thick that is tightly associated with the nucleoplasmic face of the inner nuclear membrane and appears to mediate its attachment to the underlying chromatin. In this way, the lamina appears to constitute a general framework for nuclear envelope structure while at the same time providing an anchoring site at the nuclear periphery for higher order chromatin domains.

The nuclear lamina is composed largely of one or several proteins known as lamins. Adult mammalian somatic cells typically contain three major lamins termed lamins A, B, and C (*M*, 72,000, 66,800, and 65,000 D; Gerace et al., 1978; Gerace and Blobel, 1980). In addition several minor lamins may also be present (Lehner et al., 1986; Kaufmann, 1989). Sequence and structural analysis has shown that they

are α -helical proteins and members of the larger intermediate filament protein family (McKeon et al., 1986; Fisher et al., 1986; Aebi et al., 1986). The expression of these proteins is known to be developmentally regulated (Benevento et al., 1985; Lehner et al., 1987). In mammalian early embryonic cells, including murine embryonal carcinomas, only lamin B is expressed, lamins A and C appearing relatively late during development (Stewart and Burke, 1987; Lebel et al., 1987).

The tight association with the nuclear lamina is the diagnostic feature of the interphase inner nuclear membrane. However, few details are currently known of its precise composition. Four integral proteins specific to the nuclear membranes have recently been described. One of these has been suggested to be a lamin B receptor (Worman et al., 1988). The other three (75,000, 68,000, and 55,000 D), which have been localized by immunocytochemistry to the inner membrane of rat liver nuclear envelopes, also appear to have a tight association with the lamina (Senior and Gerace, 1988). In addition, the expression of these three proteins may be developmentally regulated since a variety of rat cell lines, including normal rat kidney and buffalo rat liver contain exclusively the smallest member of the trio, p55 (Senior and Gerace, 1988).

The existence of resident integral proteins of the inner nu-

clear membrane raises the question of how these proteins are targeted to their final destination and whether specific address signals are required. For instance, soluble nuclear proteins are known to contain discrete amino acid sequences that act as nuclear localization signals (see reviews by Dingwall and Laskey, 1986 and Goldfarb, 1989). Observations on cells infected with enveloped animal viruses have indicated that at least some membrane proteins may have free access to the inner nuclear membrane. In cells infected with VSV, the envelope glycoprotein (G) may be detected in the inner nuclear membrane (Bergmann and Singer, 1983). Similarly, in Sindbis virus-infected baby hamster kidney cells, the newly synthesized spike glycoprotein also appears to have access to the inner nuclear membrane (Torrissi and Bonnatti, 1985). In the latter case, it has been demonstrated that the spike glycoprotein can return to the outer nuclear membrane and endoplasmic reticulum and can reenter the normal secretory pathway (Torrissi et al., 1987). It has been suggested that these proteins gain access to the inner nuclear membrane from their site of synthesis in the ER and outer nuclear membrane by diffusing laterally around the membrane continuities at the periphery of the nuclear pore complexes.

We have recently examined the intracellular movements of the endogenous inner membrane protein, p55 in heterokaryons. Surprisingly, this protein is able to exchange between nuclei and is the only nuclear membrane protein known to do so. The mechanisms underlying this exchange and the implications for inner membrane targeting are discussed.

Materials and Methods

Cells and Viruses

Normal rat kidney (NRK)¹ cells were maintained in DMEM containing 10% FCS (Hyclone Laboratories, Logan, UT) and penicillin/streptomycin (Gibco Laboratories, Bethesda Research Laboratories, Gaithersburg, MD). CHO cells were grown in α -MEM (α modification of Eagle's medium) containing similar supplements. P19 embryonal carcinomas and the derived cell line P19MES were maintained as described in Stewart and Burke (1987). All cells were grown at 37°C in a humidified incubator with a 7.5% CO₂ and a 92.5% air atmosphere.

VSV (Indiana serotype), a gift from Karl Matlin (Department of Anatomy and Cellular Biology, Harvard Medical School), was propagated as described by Matlin et al. (1982) with the modification that NRK cells were used in place of MDCK cells. Virus was stored frozen in aliquots at -70°C until required.

Antibodies

The mAb RL13 against p55 was a generous gift from Larry Gerace (Scripps Clinic and Research Foundation, La Jolla, CA) and has been described previously (Senior and Gerace, 1988). The antilamin antibody 1E4 (McKeon et al., 1986) was kindly donated by Frank McKeon (Department of Cellular and Molecular Physiology, Harvard Medical School). The antiprotein disulfide-isomerase antibody, RL16 (Kaetzel et al., 1987), was kindly provided by Charlotte Kaetzel (Institute of Pathology, Case Western Reserve University, Cleveland, OH). An antipeptide rabbit antibody specific for lamin A has been previously described (Burke, 1990). The mAb XB10 against lamins A and C was produced employing conventional procedures (Taggart and Samloff, 1982).

Transfections

The plasmid pHLA (Loewinger and McKeon, 1988), derived from pECE

(Ellis et al., 1986), containing a full-length human lamin A cDNA downstream from the SV-40 early promoter was a gift from Frank McKeon. It was purified by CsCl centrifugation and then introduced into cells employing the CaPO₄ method of Graham and van der Eb (1973) exactly as described by Loewinger and McKeon (1988) and Holtz et al. (1989). Any further manipulations including VSV infections and cell fusion, were initiated 20 h after transfection.

Cell Fusion

NRK cells were cocultured on glass coverslips with CHO cells or with the P19 or P19MES EC cell lines. The cells were fused with polyethylene glycol (PEG) as described by Blau et al. (1983) and Chiu and Blau (1984) and then returned to the incubator in their normal culture medium containing either 10 μ g/ml cycloheximide or anisomycin (Sigma Chemical Co., St. Louis, MO) to block protein synthesis. At various times postfusion, coverslips were fixed and processed for microscopy. For VSV-mediated cell fusion, cocultures of cells grown on coverslips were infected with VSV as previously described (Matlin et al., 1982). 3.5 h postinfection the cells were induced to fuse by a 1-min exposure to pH 5.5 MEM (bicarbonate free, buffered with morpholinoethanesulfonic acid) at 37°C (White et al., 1981). After the VSV-mediated fusion, the cells were processed in a fashion identical to those fused with PEG.

Immunofluorescence Microscopy

Cells grown on glass coverslips were fixed with formaldehyde and labeled with antibodies according to the general procedures described by Ash et al. (1977). Rhodamine- and fluorescein-conjugated secondary antibodies were obtained from Tago Inc. (Burlingame, CA). Some samples were also stained with Hoechst dye 33258 to reveal the chromosomes. Specimens were observed and photographed with a Zeiss Axiophot equipped with a X63 PlanApo objective lens.

Metabolic Labeling and Electrophoresis

Cells in 35-mm tissue culture dishes were labeled with 50 μ Ci of [³⁵S]Trans label (methionine and cysteine; ICN Radiochemicals, Irvine, CA) either in the presence or absence of 10 μ g/ml cycloheximide as described in the figure legend. Total cell lysates were fractionated by SDS-PAGE according to the methods of Laemmli (1970). The gels were subsequently fixed in 10% TCA, impregnated with En³Hance (New England Nuclear, Boston, MA), dried, and exposed to x-ray film at -70°C. Exposed films were scanned using a scanning densitometer (GS300; Hoefer Scientific Instruments, San Francisco, CA).

Results

Study of the targeting of proteins to the inner nuclear membrane is complicated by the fact that there are no posttranslational modifications which are known to occur exclusively at this site. Consequently there is no simple way to detect biochemically the arrival of a protein at the inner nuclear membrane. In view of this, as a first approach to analyze protein targeting to the inner nuclear membrane, we have examined the exchange between nuclei of an inner nuclear membrane protein, p55, in heterokaryons by means of immunofluorescence microscopy using species-specific mAbs.

The murine mAb RL13 has been described previously (Senior and Gerace, 1988). In rat liver, it recognizes three integral inner nuclear membrane proteins, while in a variety of rat cell lines, including NRK cells, RL13 detects only the smallest of these three proteins, p55 (see Senior and Gerace, 1988, Fig. 5). A notable, and for this study essential feature of this antibody is that its reactivity is restricted exclusively to rat cells (Senior and Gerace, 1988; see also Figs. 1 and 3). We have therefore used NRK cells, to construct heterokaryons with both hamster cells and mouse cells, to determine whether p55 can exchange between nuclei. Initially we constructed NRK/CHO cell heterokaryons employing the

1. Abbreviations used in this paper: BRL, buffalo rat liver; NRK, normal rat kidney; PDI, protein disulfide isomerase.

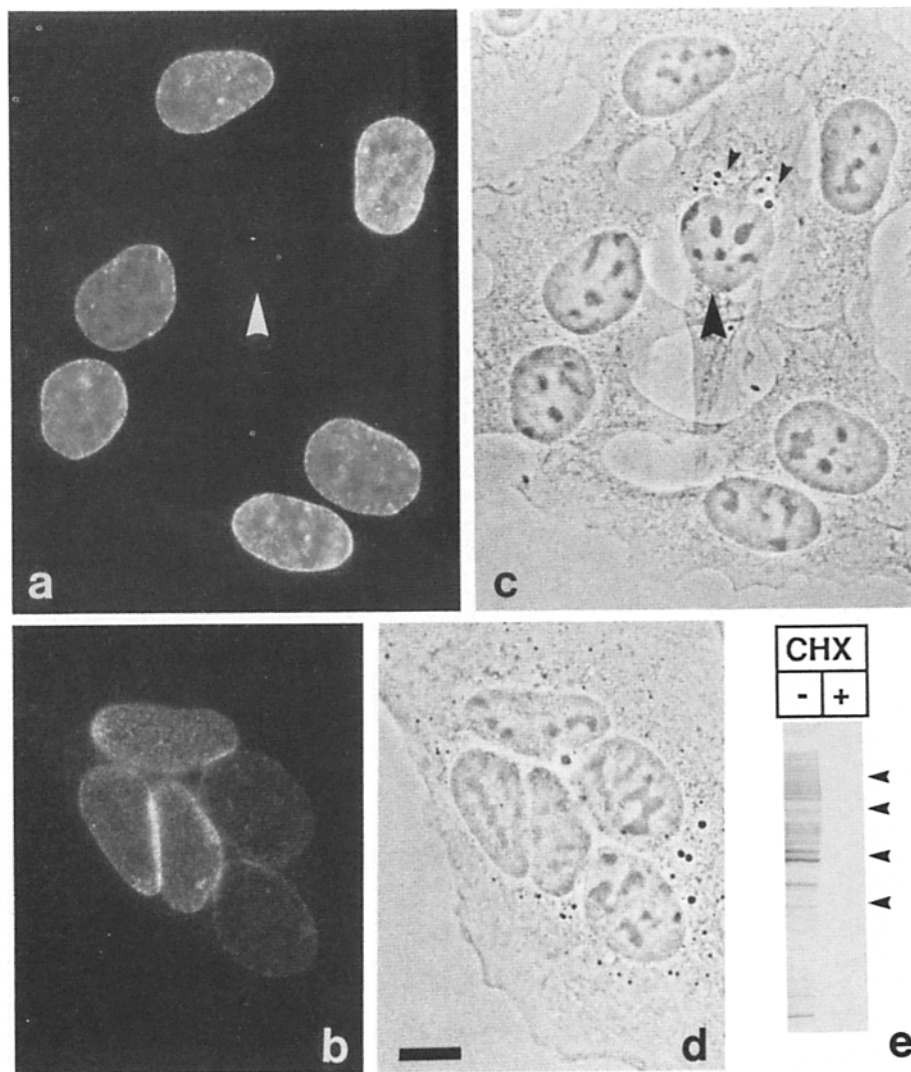


Figure 1. Immunofluorescence microscopy of a co-culture of NRK cells and CHO cells (*large arrowhead*) using the antibody RL13, recognizing p55 (*a*). The CHO cell which is negative for p55 has distinctive cytoplasmic inclusions (*c*, *small arrowheads*) visible under phase contrast optics. (*b* and *d*) show a similar culture maintained in medium containing cycloheximide (CHX, 10 $\mu\text{g/ml}$) for 3 h after polyethylene glycol-mediated cell fusion. The heterokaryon shown must be derived from both NRK and CHO cells since all five of its nuclei are positive for p55 (*b*), while at the same time it possesses the refractile inclusions characteristic of CHO cells (*d*). *e* shows the effects of cycloheximide on [^{35}S]methionine incorporation into total cell proteins over the time course of the experiment detected by gel electrophoresis and fluorography. In the presence of 10 $\mu\text{g/ml}$ cycloheximide (+) total protein synthesis is reduced by 94% over untreated controls (-). The small arrowheads refer from top to bottom to molecular weight markers for 92, 68, 45, and 31 kD, respectively. Bar, 10 μm .

polyethylene glycol fusion method. In mixed cultures, CHO cells can be easily identified by virtue of their phase dense cytoplasmic inclusions, probably lipid droplets, uniformly absent from NRK cells, but are not reactive with the RL13 antibody (Fig. 1). After fusion, heterokaryons must, therefore contain the cytoplasmic inclusions and at least some of the nuclei must label with RL13 (Fig. 1).

To follow the fate of p55 in heterokaryons, immediately following fusion the cells were returned to their normal growth medium and incubated at 37°C for several hours in the presence of 10 $\mu\text{g/ml}$ cycloheximide (or anisomycin, data not shown) to block new protein synthesis. The cells were then processed for immunofluorescence microscopy using RL13. As can be seen in Fig. 1 *b*, within 3 h of fusion, all of the nuclei within heterokaryons were found to contain at least some p55 associated with their envelopes. This result implies that this protein may redistribute from the NRK to CHO nuclei since it occurs in the absence of protein synthesis (Fig. 1 *e*) and must therefore involve p55 preexisting at the time of fusion. This redistribution is not a general feature of nuclear envelope proteins since the nuclear lamins never exchange between nuclei in heterokaryons even after incubation for 20 h. This was demonstrated using the mAb 1E4, which can discriminate between rat and hamster lamins A

and C (Fig. 2). Borer et al. (1989) have reported similar results in mouse/chick heterokaryons where no exchange of lamins could be detected as long as 72 h after fusion. This result, and those described below indicate that the observed redistribution of p55 is not simply due to fusion of nuclear envelopes. Furthermore, since transfection studies have shown that CHO nuclear envelopes will readily accumulate heterologous lamins (Loewinger and McKeon, 1988), it must be concluded that once stably integrated into the nuclear lamina, these proteins are never free to leave the nucleus until the nuclear envelope undergoes mitotic disassembly. Under the experimental conditions used in this study, we have never observed mitotic figures in heterokaryons, ruling it out as a possible major mechanism of nuclear membrane protein redistribution.

While these experiments using CHO/NRK hybrids suggest that p55 can move between nuclei, they suffer from the drawback that the CHO and NRK nuclei cannot be unequivocally distinguished microscopically. We therefore decided to construct rat/mouse heterokaryons for subsequent experiments. Mouse nuclei are easily identified in these hybrid cells after staining with Hoechst dye #33258 since they contain discrete regions of chromatin, which are heavily labeled with this reagent. The rat nuclei on the other hand exhibit

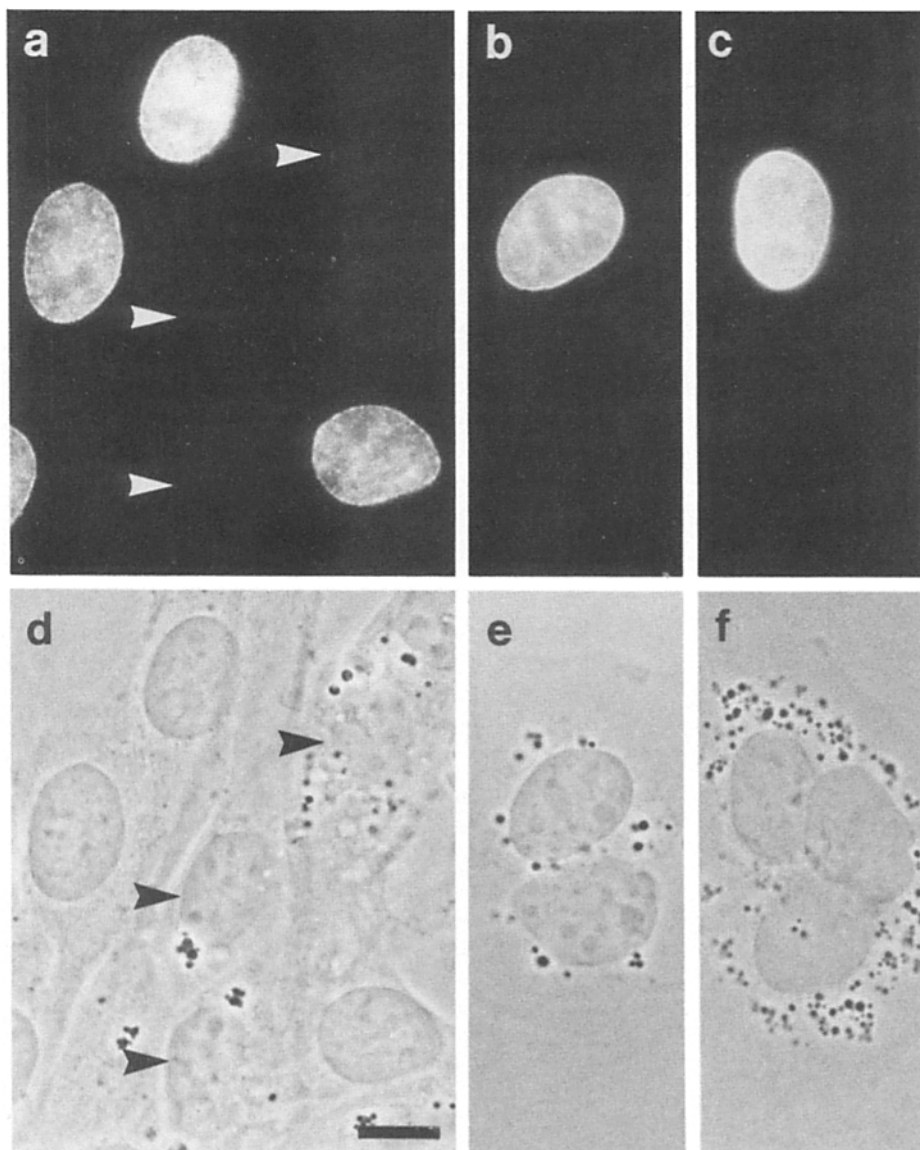


Figure 2. Immunofluorescence microscopy of cocultures of NRK and CHO cells using the mAb IE4, which recognizes rat but not hamster lamins A and C. *a* and *d* show an unfused culture with the IE4-negative CHO nuclei indicated by arrowheads. Note the presence of the cytoplasmic inclusions in the CHO cells in *d*. Heterokaryons are shown 3 h (*b* and *e*), and 20 h (*c* and *f*) after PEG mediated cell fusion. During the time course of the experiment, in which protein synthesis was inhibited with cycloheximide, there was no discernable movement of lamins between nuclei. Bar, 10 μ m.

relatively uniform staining (Fig. 3 *b*). To avoid any possible artifactual redistribution of p55 induced by PEG, we constructed these rat/mouse heterokaryons by first infecting cocultures of cells with VSV and then using the viral G protein expressed at the cell surface to promote plasma membrane fusion by brief exposure to medium at pH 5.5. It is worth pointing out, however, that in reality both of these very different fusion protocols were ultimately found to give identical results in terms of p55 redistribution.

Rat/mouse heterokaryons were constructed using NRK cells and P19MES fibroblasts, a derivative of the murine embryonal carcinoma cell line P19. When these heterokaryons were examined by immunofluorescence microscopy at various times after fusion, and in the absence of new protein synthesis, it was clear that p55, visualized with the rat specific RL13 mAb was appearing within mouse nuclear envelopes in heterokaryons (Fig. 4). That this was due to redistribution of p55 from rat to mouse nuclei was indicated by the fact that the intensity of labeling of the rat nuclear envelopes dimin-

ished while there was a concomitant increase in the labeling of the mouse nuclear envelopes (Fig. 4 *g*). Equilibration of p55 between rat and mouse nuclei in dikaryons typically occurred over a period of ~ 3 –6 h. During this period there was no evidence of nuclear fusion, consistent with observations from many previous studies employing heterokaryons (e.g., Harris, 1975; Bolund, et al., 1969; Scheer et al., 1983; Blau et al., 1983; Borer et al., 1989). Since there are currently no antibodies available which can distinguish between rat and mouse lamins, it was not possible to determine whether these proteins underwent any redistribution. However, in the light of the CHO experiments (above) and others to be discussed, it is considered highly unlikely.

Additional experiments were carried out in which heterokaryons were constructed from NRK and P19 cells, the undifferentiated parent cell line of P19MES. Surprisingly, even after extended incubation, little or no p55 could be detected within the P19 nuclear envelopes (Fig. 5), in marked contrast to the results described above with the P19MES het-

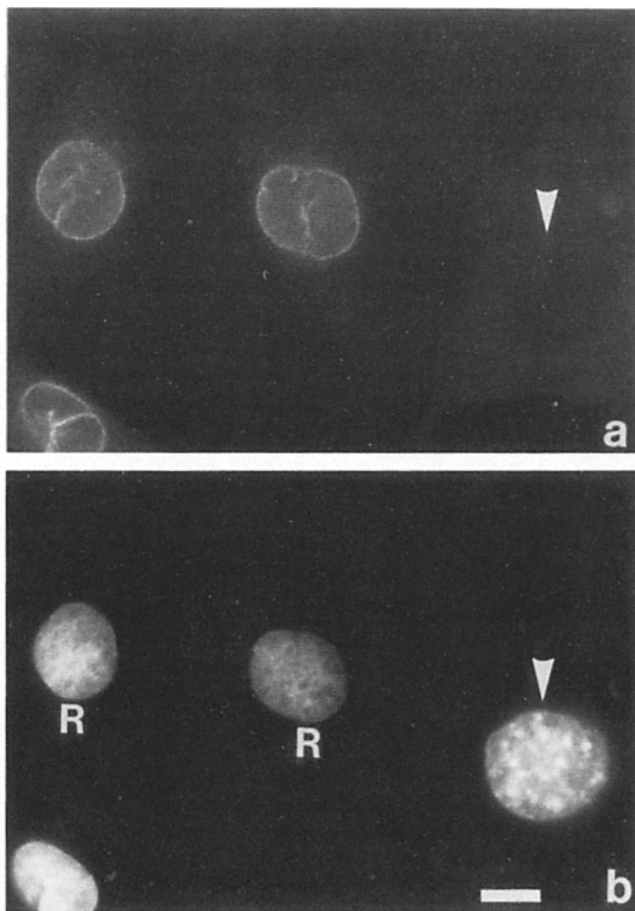


Figure 3. Immunofluorescence labeling with RL13 (*anti-p55*) of cocultures of VSV infected NRK and P19MES cells before low pH induced fusion (*a*). The mouse (*P19MES*) nuclei (*arrowheads*) are easily distinguished from the p55-positive rat nuclei (*R* in *b*) by their punctate appearance when labeled with Hoechst dye 33258 (*b*). Bar, 10 μm .

erokaryons. It seems unlikely that access of rat p55 to the inner nuclear membrane should be restricted in P19 nuclei but not in those from the derived cell line P19MES. A more plausible explanation would be that p55 does indeed have access to the P19 inner nuclear membrane but that these lack some component with which this protein specifically associates and as a consequence p55 does not concentrate in this membrane. Nuclear envelopes from these two cell lines do indeed exhibit major differences in their protein composition. We have previously shown that while the P19MES cells possess the normal adult complement of three nuclear lamins A, B, and C, the parent line P19 contains only lamin B (Stewart and Burke, 1987). Since p55 exhibits a tight *in vitro* association with the nuclear lamina (Senior and Gerace, 1988) this could in principal occur partly via lamins A and C. This in turn could account for p55 accumulation in P19MES but not P19 nuclei in the heterokaryons. Such a simple hypothesis is easily testable since it would be predicted that P19 nuclei from cells transfected with lamins A and/or C would be rendered competent to accumulate rat p55 in heterokaryons. We have transiently transfected cocultures of P19 and

NRK cells with a cDNA encoding human lamin A, and from these constructed heterokaryons. We then looked by double-label immunofluorescence microscopy for p55 accumulation in P19 nuclei, which also contain the human lamin detected with an antipeptide antibody against lamin A. We already know that heterologous lamins are correctly targeted to P19 nuclear envelopes and undergo correct posttranslational processing (Collard and Raymond, 1990; Horton, H., and B. Burke, manuscript in preparation). As shown in Fig. 6, it was consistently observed that P19 nuclei positive for lamin A, and therefore derived from transfected cells, accumulate p55 in heterokaryons, while their lamin A negative neighbors do not. As a control for antibody specificity, Fig. 6, *a-d* shows that in unfused transfected cultures, only the NRK nuclei are labeled by RL13 (*anti-p55*). The P19 nuclei are unlabeled by RL13 irrespective of whether or not they are labeled by the antilamin A antibody. As a final point, it is clear from Fig.

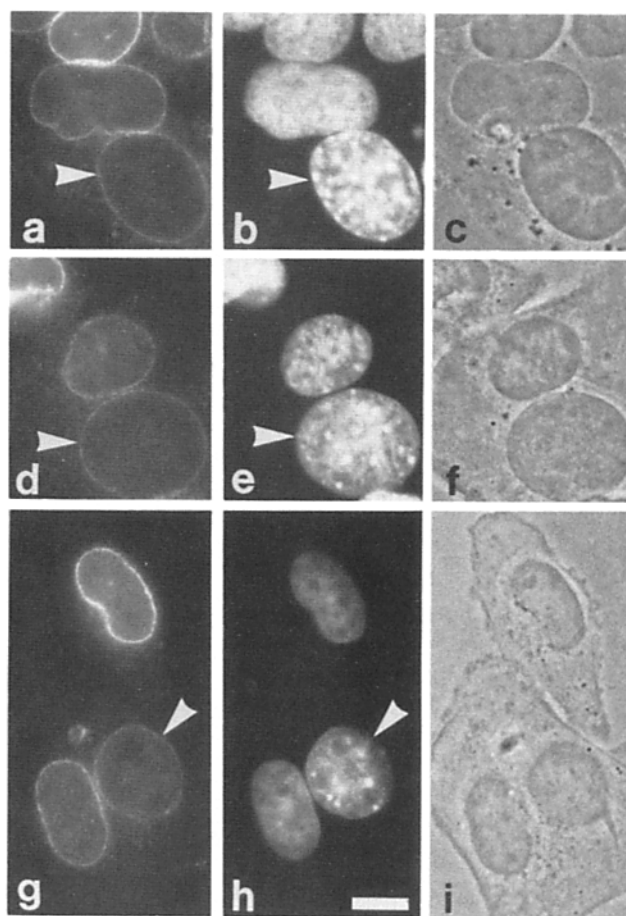


Figure 4. P19MES/NRK heterokaryons 3 (*a-f*) and 6 h (*g-i*) after VSV-mediated cell fusion. Immunofluorescence labeling with RL13 (*anti-p55*) is shown in the left column (*a*, *d*, and *g*), whereas the same fields labeled with Hoechst dye 33258 to identify mouse nuclei (*arrowheads*) are shown in the middle column (*b*, *e*, and *h*). The corresponding fields visualized by phase contrast microscopy are in the right column (*c*, *f*, and *i*). In *a-c* and *d-f* the mouse nuclei shown are in heterokaryons containing a two- to threefold excess of rat nuclei. In *g-i* the mouse nucleus shares the same cytoplasm with a single rat nucleus. Bar, 10 μm .

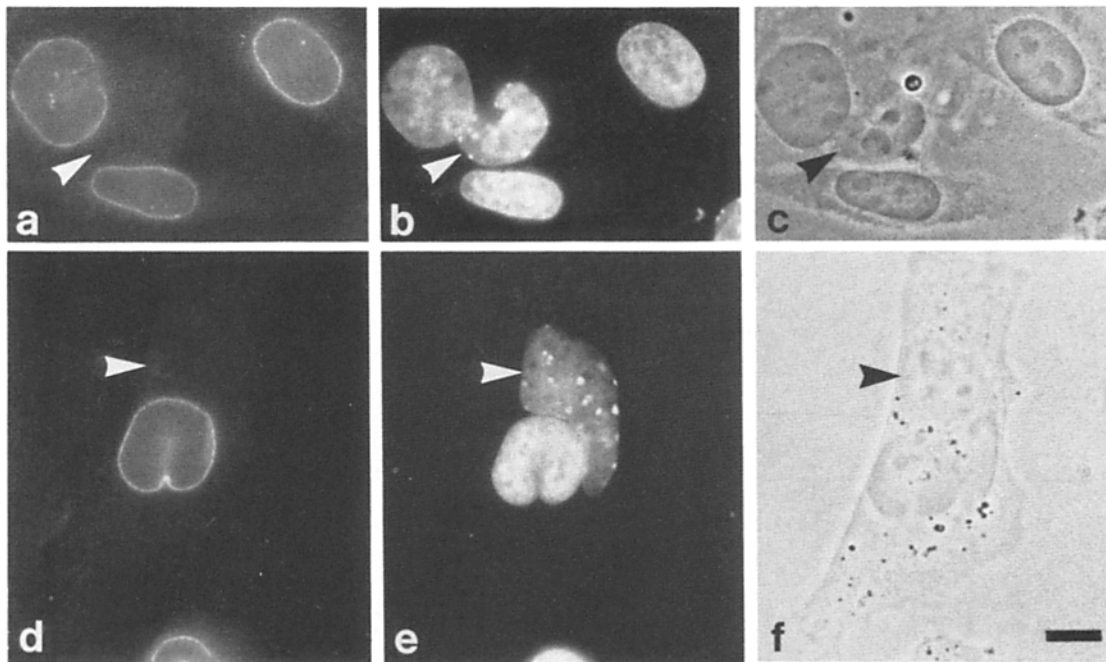


Figure 5. Immunofluorescence microscopy of cocultures of P19 and NRK cells either before (*a-c*) or 3 h after (*d-f*) VSV-mediated fusion. Labeling with RL13 is shown in *a* and *d* while mouse (*P19*) nuclei are identified (*arrowheads*) by staining with Hoechst dye in *b* and *e*. Corresponding phase contrast images are shown in *c* and *f*. Little p55 appears in the P19 nuclear envelopes in heterokaryons in contrast to the marked accumulation seen in the P19MES nuclear envelopes shown in Fig. 4. Bar, 10 μm .

6 *f* that as in the NRK/CHO heterokaryons (Fig. 2) there is no exchange of lamins between nuclei during the time course of the experiment.

Since p55 has been shown to be an integral membrane protein (Senior and Gerace, 1988), the question arises as to how it can exchange between nuclei in heterokaryons. One possibility is that it might occur via fusion of heterologous ER elements, which are in direct continuity with the nuclear membranes and which may then act as membranous bridge between nuclei. In an effort to address this question we have attempted to determine whether an ER-specific protein can exchange between rat and mouse ER in heterokaryons. To do this, we observed the redistribution of the luminal ER enzyme, protein disulfide isomerase (PDI), from rat to mouse ER using a rat-specific mAb. To distinguish rat from mouse ER we looked exclusively for the appearance of rat PDI in mouse nuclear envelopes, or more accurately the perinuclear space, identified by Hoechst staining of the parent nuclei. As shown in Fig. 7, within 1 h of fusion, PDI labeling of rat and mouse nuclear envelopes appeared at comparable intensity suggesting rapid intermixing of ER components.

Discussion

In this paper we have described immunofluorescence observations which suggest that a nuclear envelope protein, p55, originally described by Senior and Gerace (1988), appears to be able to redistribute between nuclei in heterokaryons. This redistribution is unlikely to be due to mitosis-dependent rearrangement of nuclear envelope components since mitosis occurs at only an extremely low frequency, if at all, in these heterokaryons. Furthermore, no redistribution of

nuclear lamins is observed, even over extended periods. Neither is it likely to be due to nuclear fusion since this has never been observed in many other somatic cell hybridization studies involving interphase cells (e.g., Harris, 1975; Bolund et al., 1969; Scheer et al., 1983; Blau et al., 1983; Borer et al., 1989) and is difficult to reconcile with our own results obtained with the embryonal carcinoma stem cells and their derivatives. A further possible interpretation of the results presented here is that the RL13 antibody might in fact detect a rat-specific secondary modification of p55, and that what we were really observing in our experiments was modification of mouse p55 such that it could be bound by RL13. However, such a scenario does not easily account for the effect of lamin A in the undifferentiated embryonal carcinomas (Fig. 6) nor the fact that the appearance of labeling of mouse nuclei in heterokaryons is coincident with the decline in labeling of rat nuclei (Fig. 4 *g*). The most reasonable interpretation of the results presented here and one which will account for all of the observations, is that p55 is capable of shuttling between the nucleus and the cytoplasm and that this leads to equilibration of p55 between nuclei in heterokaryons. Such interphase shuttling has been observed for a number of other apparently 'resident' nuclear proteins (see Appels and Ringertz, 1975, and Borer et al., 1989 for references). What is unique in the case of p55, however, is that it is the only integral protein of the inner nuclear membrane suggested to be capable of this behavior. This in turn presents a number of implications concerning the mechanisms of protein targeting to and sequestration in this particular membrane.

Exchange between nuclei obviously requires that p55 move bidirectionally between the nuclear interior and the

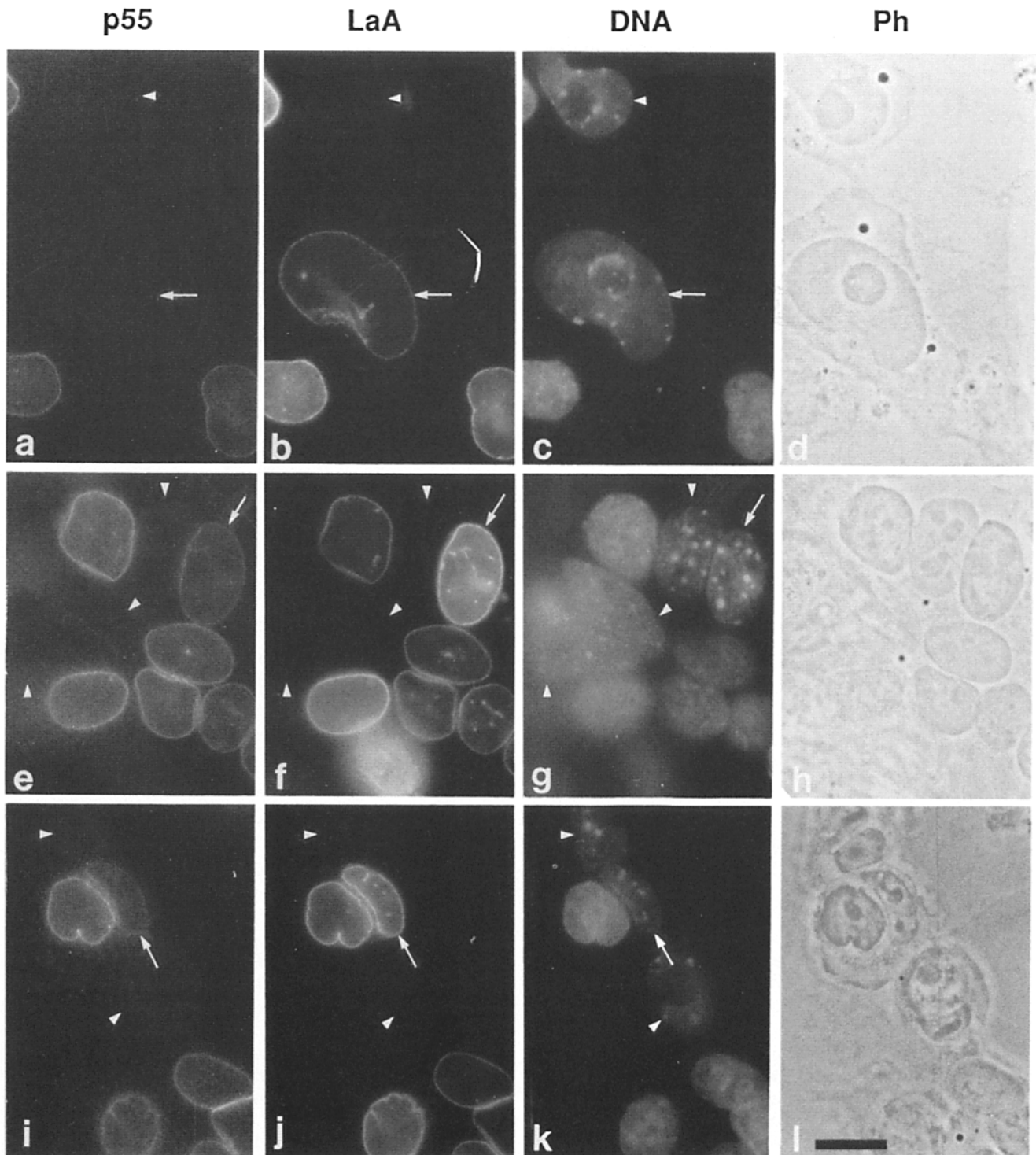


Figure 6. Double immunofluorescence microscopy of cocultures of P19 and NRK cells transfected with a cDNA encoding human lamin A. 20 h posttransfection, the cells were infected with VSV as described. Cultures were then fixed and processed for microscopy either before (*a-d*) or 3 h after (*e-i*) low pH induced fusion and incubation in the presence of cycloheximide (10 $\mu\text{g/ml}$). Cells were labeled with RL13 against p55 (*a*, *e*, and *i*; *p55* column) and a rabbit antipeptide antibody specific for lamin A (*b*, *f*, and *j*; *LaA* column). Secondary antibodies were fluorescein-conjugated goat anti-mouse IgG and rhodamine conjugated goat anti-rabbit IgG, respectively. Also shown are the same fields stained with Hoechst dye (*c*, *g*, and *k*; *DNA* column) to identify mouse nuclei (*arrows* and *arrowheads*). Phase-contrast images are in (*d*, *h*, and *l*; *Ph* column). In each of the panels, the arrows indicate P19 nuclei which contain human lamin A. After cell fusion these were found to be competent to accumulate p55 (*e* and *i*). The arrowheads indicate P19 nuclei which do not contain the transfected lamin. In *e-h*, all of the indicated nuclei reside within the same cytoplasm. Bar, 10 μm .

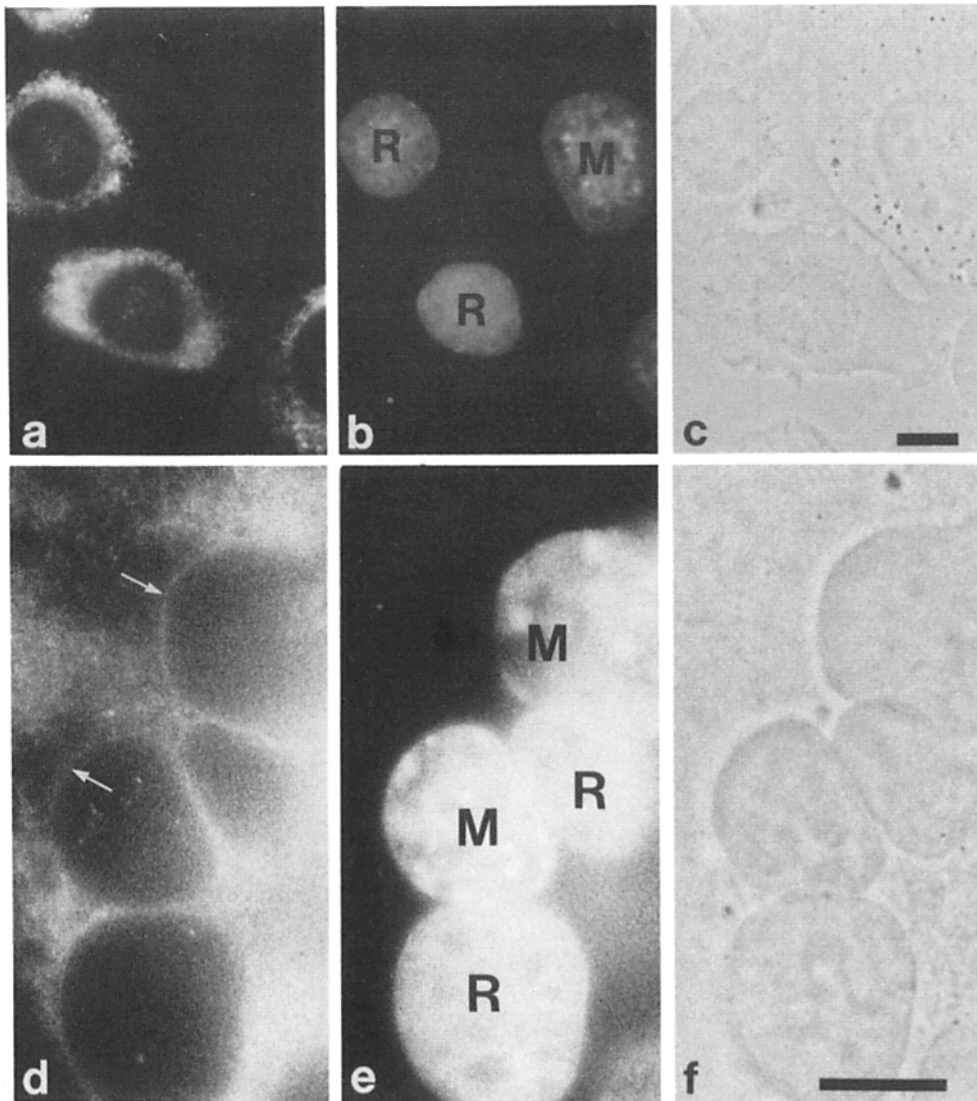


Figure 7 Immunofluorescence microscopy of cocultures of P19MES and NRK cells either before (*a-c*) or 1 h after (*d-f*) VSV-mediated fusion. The cells were labeled with RL16, an mAb against protein disulfide isomerase which recognizes the rat but not the mouse form of the enzyme (*a* and *d*). Mouse (*M*) and rat (*R*) nuclei are identified with Hoechst dye in *b* and *e*. Corresponding phase contrast images are in *c* and *f*. The arrows (*d*) indicate mouse nuclear envelopes within a heterokaryon, which appear to contain rat PDI. Bars, 10 μ m.

cytoplasm and this will in all likelihood involve the nuclear pore complexes. For soluble proteins with molecular mass >40–60 kD, the size limit for passive diffusion through the nuclear pore complex (Paine, 1975, see also review by Dingwall and Laskey, 1986), nuclear import requires a specific nuclear localization signal, typically a discrete sequence of mainly basic amino acids (Kalderson et al., 1984). If such a protein were capable of bidirectional movement across the nuclear envelope it is possible that reexport would also be signal mediated, although no such export signals on proteins have so far been described. A number of recent studies have shown that signal-mediated import of soluble proteins, and indeed export of mature ribonucleoproteins, takes place through a central channel or transporter at a radial distance of \sim 45 nm from the membrane continuities towards the periphery of the pore complex (Feldherr et al., 1984; Dworetzky and Feldherr, 1988; Akey and Goldfarb, 1989). This geometry seems to preclude the idea that integral proteins destined for the inner nuclear membrane use the same import machinery as soluble karyophilic proteins, assuming that they remain membrane associated. The possibility then arises that the pore complex possesses a different gating mecha-

nism for membrane proteins. An alternative, however, is that the pore complex may not be a selective barrier at all for many membrane proteins, but may allow free passage around the continuities between the inner and outer nuclear membranes, a suggestion supported by the observations of Bergmann and Singer (1983) and Torrisi et al. (1987) on viral membrane glycoproteins. If the latter were the case, sequestration of certain proteins in the inner nuclear membrane would have to be mediated via a specific interaction with other nuclear components. Our data suggests that this may be what occurs with p55, where expression of lamin A favors the accumulation of this protein within the nuclear envelope in EC cell heterokaryons. This observation is consistent with the known solubility properties of p55, which suggests that it indeed interacts with the nuclear lamina. It is also consistent with the notion that the lamina may act as a general framework for the organization of other nuclear envelope components (Gerace et al., 1984). While our data implicate lamin A as a potential p55 binding protein, they by no means prove it. It could certainly be envisaged, for instance, that p55 actually interacts with lamin B or indeed some other nuclear component and that expression and assembly of lamin

A merely causes a reorganization of the lamina such that a p55-binding site becomes available. Further in vivo and in vitro studies will be required to clarify this point.

Exchange of p55 between nuclei in heterokaryons raises a number of other issues. Since by all accepted criteria p55 is an integral membrane protein (e.g., resistance to extraction by NaOH; Senior and Gerace, 1988), this exchange is likely to be membrane mediated. The most reasonable mechanism involves the endoplasmic reticulum, which is known to be continuous with the outer nuclear membrane, acting as some form of "bridge" between the nuclei in heterokaryons. This clearly requires that the ER from each fusion partner be in at least some form of communication with the other. This could involve anything from an exchange of vesicles to complete fusion of the two ERs. That the rat and mouse ERs in the heterokaryons are in communication is supported by the observations on the exchange of PDI shown in Fig. 7. Since PDI possesses the ER retention signal KDEL at its carboxy terminus (Munro and Pelham, 1987), this communication could simply entail recycling of PDI (and p55?) containing vesicles between the two ERs via an intermediate compartment (Armstrong and Warren, 1990). In all likelihood, however, direct fusion of ER elements does take place in heterokaryons. Observations made both in vitro (Dabora and Sheetz, 1988) and in vivo in single cells (Lee and Chen, 1988), have shown that the ER, far from being a static structure is a highly fluid network of tubular elements that are continuously extending along microtubules and breaking and fusing with each other. Such activity seems likely to continue following cell hybridization.

In summary, we have shown that an integral protein, p55, of the inner nuclear membrane can exchange between nuclei in rat/mouse and rat/hamster heterokaryons. This is the only example to date of such exchange and suggests that the interphase nuclear envelope may be a far more dynamic structure than had hitherto been anticipated. It had previously been suggested that p55 is associated with nuclear lamina and may serve to anchor this structure to the inner nuclear membrane (Senior and Gerace, 1988). Experiments using an embryonal carcinoma stem cell line as a fusion partner add credence to this notion since they demonstrate that concentration of this protein in the inner nuclear membrane is contingent upon the presence of nuclear lamin A. In terms of protein sorting, our observations and those of others (Bergmann and Singer, 1983; Torrisi et al., 1987), suggest that in certain cases, specific signals may not be necessary, or at least sufficient to cause accumulation of proteins in the inner nuclear membrane. Instead, sequestration in the inner nuclear membrane may involve interactions with other nuclear components. Whether this is true for all inner nuclear membrane proteins clearly requires further analysis.

We would like to thank Morrie Birnbaum, Larry Gerace, Karl Matlin, Frank McKeon and Isabel McMorrow for helpful discussion; Larry Gerace, Charlotte Kaetzel and Frank McKeon for generous gifts of antibody; Karl Matlin and Kristin Shotwell for VSV stocks.

This work was supported by grants from the National Institutes of Health and from the Lucille P. Markey Charitable Trust.

Received for publication 4 June 1990 and in revised form 29 August 1990.

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