Mobility of cytochrome P450 in the endoplasmic reticulum membrane

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ABSTRACT Cytochrome P450 2C2 is a resident endoplasmic reticulum (ER) membrane protein that is excluded from the recycling pathway and contains redundant retention functions in its N-terminal transmembrane signal/anchor sequence and its large, cytoplasmic domain. Unlike some ER resident proteins, cytochrome P450 2C2 does not contain any known retention/retrieval signals. One hypothesis to explain exclusion of resident ER proteins from the transport pathway is the formation of networks by interaction with other proteins that immobilize the proteins and are incompatible with packaging into the transport vesicles. To determine the mobility of cytochrome P450 in the ER membrane, chimeric proteins of either cytochrome P450 2C2, its catalytic domain, or the cytochrome P450 2C1 N-terminal signal/anchor sequence fused to green fluorescent protein (GFP) were expressed in transiently transfected COS1 cells. The laurate hydroxylase activities of cytochrome P450 2C2 or the catalytic domain with GFP fused to the C terminus were similar to the native enzyme. The mobilities of the proteins in the membrane were determined by recovery of fluorescence after photobleaching. Diffusion coefficients for all P450 chimeras were similar, ranging from 2.6 to 6.2×10^{-10} cm²/s. A coefficient only slightly larger (7.1×10^{-10} cm²/s) was determined for a GFP chimera that contained a C-terminal dilysine ER retention signal and entered the recycling pathway. These data indicate that exclusion of cytochrome P450 from the recycling pathway is not mediated by immobilization in large protein complexes.

The general rules governing mechanisms of subcellular localization of membrane proteins remain poorly understood. Proteins in the membranes of the endoplasmic reticulum (ER), Golgi apparatus, plasma membrane, and transport and storage vesicles in the transport pathway are initially inserted into the membrane of the ER. The existence of positive or negative (retention) targeting signals and the formation of large complexes or networks that immobilize the protein and are incompatible with inclusion in transport vesicles have been proposed as mechanisms for targeting the proteins to their ultimate location (reviewed in ref. 1). Several lines of evidence suggest that a bulk-flow mechanism is a default pathway of transport from the ER, but the transport of secreted proteins at rates considerably higher than that of the bulk flow indicates that positive signals for efficient transport exist. Many ER resident proteins are transported from the ER and then retrieved by a retention signal-mediated process. The sequences KDEL and KKXX have been identified as retention/retrieval signals for the soluble and membrane-bound ER proteins, respectively, and deletion of these sequences results in transport out of the ER, supporting bulk flow as a default

pathway (1–3). Similar signals have not been detected for membrane proteins that are restricted to the ER without retrieval. Likewise, discrete signals specifying retention in the Golgi have not been identified; relatively long hydrophobic transmembrane segments and flanking regions of the proteins are important for retention (4, 5). Subsequently, it was suggested that retention of Golgi membrane proteins is mediated by “kin” recognition and formation of large oligomeric structures, the immobility of which prevents further transport (6, 7). Similarly, it also has been proposed that oligomerization and network formation immobilize and/or prevent transport of resident ER membrane proteins that do not have defined protein retention signal motifs (8, 9).

Cytochromes P450 (P450) are resident ER membrane proteins that are retained directly in the ER and do not undergo recycling through the pre-Golgi compartment (10, 11). P450 is targeted to the ER by an N-terminal signal sequence, which is not cleaved; it also serves to anchor the protein to the membrane (12, 13). Redundant signals for retention are present in P450 because both the N-terminal signal/anchor and the catalytic domain cause retention of heterologous proteins in the ER (11, 14, 15). Specific sequence motifs have not been identified as retention signals, and multiple regions of the catalytic domain appear to contribute to the retention function (11, 14). These negative results are consistent with the hypothesis that the mechanism of P450 retention in the ER may be related to formation of a network or aggregate, rather than requiring specific sequence motifs as signals. Furthermore, P450 is known to form oligomers (16) and, additionally, it forms molecular complexes with its two partners, cytochrome P450 reductase and cytochrome b₅, both ER membrane proteins (17), which potentially could result in a large network or an aggregate, thus immobilizing P450.

The mobilities of membrane proteins have been estimated by measuring the recovery of fluorescence in a photobleached area that results from the movement of unbleached molecules into the bleached area (18, 19). Until recently, such studies have been largely restricted to cell-surface proteins, which can interact directly with fluorescent antibodies, whereas similar studies on intracellular proteins required microinjection of fluorescent antibodies. Introduction of the green fluorescent protein (GFP) as a fluorescent tag for subcellular localization studies (20) permits measurements of the mobilities of intracellular proteins. This technique has been applied to the Golgi-specific proteins, which were found to have mobilities greater than those of plasma-membrane proteins, arguing strongly against their retention in the Golgi via immobile network formation (21). Mobilities of ER resident proteins

Abbreviations: P450, cytochrome P450; ER, endoplasmic reticulum; GFP, green fluorescent protein; EGFR, epidermal growth factor receptor; *D*, diffusion coefficient.

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have not been measured in living cells, but the studies with the Golgi proteins indicate that the photobleaching/fluorescence recovery technique should be applicable for ER proteins as well.

In this report, the mobilities of GFP chimeric proteins containing either intact P450, its N-terminal signal/anchor sequence, or its catalytic cytoplasmic domain have been measured by photobleaching/fluorescence recovery. Lateral mobilities of all three chimeras were similar to each other and to a protein that enters the transport pathway, which is inconsistent with immobilization by formation of a network as a mechanism of P450 ER retention.

MATERIALS AND METHODS

Plasmid Constructions. Chimeric P450/GFP genes were constructed by insertion of P450 coding sequences into the vector pEGFP-N1 (CLONTECH), which contains the GFP coding sequence. To make plasmid C1(1–29)/GFP, which contains the 29 N-terminal amino acids of P450 2C1 fused to the N terminus of GFP, a *Bgl*II–*Hind*III fragment of the plasmid pCMVC1 (15) was inserted into the *Bgl*II–*Hind*III site of the EGFP-N1 vector. To construct plasmid C2(1–490)/GFP, which encodes full-length P450 2C2 fused to the N terminus of GFP, a *Bam*HI recognition site was engineered at the C terminus of P450 in plasmid pC2A (22) by single-strand DNA mutagenesis with mutagenic primer 5′-AGCTTCATT-CCTGTCGGAGGAACGGATCCTCACGGGATGCCATG-3′. A *Kpn*I–*Bam*HI fragment from the modified vector was inserted into *Kpn*I–*Bam*HI-digested pEGFP-N1. Plasmid OEC2/GFP encodes a chimeric protein with the fusion of the chimeric protein OEC (14) to the N terminus of GFP. OEC contains the epidermal growth factor receptor (EGFR) transmembrane domain as an N-terminal insertion signal fused to the cytoplasmic domain of P450 2C2 (aa 22–490). This plasmid was constructed by substituting a *Bgl*II–*Hind*III fragment of the plasmid C2(1–490)/GFP with the corresponding *Bgl*II–*Hind*III fragment of the plasmid OEC.

To produce a chimeric GFP protein containing a dilysine ER retention motif, the signal sequence of EGFR was fused at the N terminus of GFP to provide targeting to the ER and C2(1–29) modified to have Lys at –3 and –4 was fused at the C terminus. When fused to the C terminus of heterologous proteins, C2(1–29) loses ER retention function (14). To construct plasmid GFP/C1(1–29), which encodes a chimeric protein with the P450 N-terminal 29 amino acids fused at the C terminus of GFP, a fragment encoding P450(1–29), with *Not*I and *Bsr*GI sites introduced by the primers, was amplified by using PCR with 5′-GGCGCGTGATCATGGATCCTGTGGTGGTGCTG-3′ as the 5′ primer, 5′-GGAGCGATGCGGCCGCTAGAGTCGACCTGCAGGCATGCA-3′ as the 3′ primer and plasmid ECO (14) as a template. The fragment obtained was digested with *Bsr*GI and *Not*I and inserted into the corresponding sites of the EGFP-N1 vector. To construct plasmid EGFR/GFP/C1(1–29), which contains the EGFR signal sequence attached to the N terminus of plasmid GFP/C1(1–29), EGFR/TZ (14) was cut with *Sty*I, filled in with *Escherichia coli* DNA polymerase I and Klenow fragment; after digestion with *Kpn*I, the 260-bp fragment containing the EGFR sequence was isolated and inserted into *Kpn*I/*Sma*I-digested GFP/C1(1–29). To make plasmid EGFR/GFP/C1(1–29)KK (which encodes EGFR/GFP/C1(1–29) modified by introduction of Lys residues at position –3 and –4 from the C terminus of the chimeric protein), PCR amplification with GGCGCGTGATCATGGATCCTGTGGTGGTGCTG as the 5′ primer, GGAGCGATGCGGCCGCTAGAGTCGCTTCTTAGGCATGCAAGCTTATCG as the 3′ primer and plasmid GFP/C1(1–29) as a template was performed. The PCR product was digested with *Bsr*GI and *Not*I and inserted

into EGFR/GFP/C1(1–29) from which the *Bsr*GI–*Not*I fragment had been deleted.

Cell Culture and Transfection. Cell culture media and antibiotics were from Life Technologies (Gibco/BRL) and calf serum was from Sigma. COS1 cells were grown and transfected as described (10). Expressions in bacteria and insect cells were performed as described (23).

Immunoprecipitation of Expressed Proteins. Forty-eight hr after transfection, cells were incubated for 4 hr with 50 μ Ci/ml (1 Ci = 37 GBq) *trans* 35 S label in methionine- and cysteine-free minimal essential medium. Radioactive proteins were immunoprecipitated and analyzed by using SDS/PAGE as described (10).

Determination of Laurate Hydroxylase Activity. Laurate hydroxylase activity was assayed in whole-cell lysates of transfected COS1 cells and of infected insect cells, and lauric acid metabolites were separated by HPLC as described (23, 24), except that the reaction was incubated for 15 min.

Quantification of C2(1–490)/GFP Protein by Fluorometric Assay. Expression of C2(1–490)/GFP in insect cells and of wild-type GFP in *E. coli* was performed, using baculoviral vector pFASTBACT and vector pINIII, respectively (23; B. Doray, C.C., and B.K., unpublished data). A GFP standard curve was generated by using known amounts of recombinant GFP purified from *E. coli* (B. Doray, C.C., and B.K., unpublished data) and measuring the fluorescence in a FluoroMax-2 (Industrial Science Associates, Ridgewood, NY) using a 490-nm excitation filter and a 510-nm emission filter. Lysates of mock-transfected and C2(1–490)/GFP-transfected cells were prepared exactly as for the activity assay. The relative fluorescence intensity of C2(1–490)/GFP was obtained by subtracting the fluorescence of mock-transfected cells and used to determine the GFP concentration from the standard curve.

Analysis of Subcellular Localization by Fluorescent Microscopy. Twenty-four hours after transfection, cells were replated into 35-mm culture dishes containing coverslips and incubated 24 hr longer. Cells were fixed with 4% paraformaldehyde and were examined with a Zeiss Universal microscope equipped with epi-illumination optics as described (10).

Photobleaching Recovery Assay. Twenty-four hours after transfection, COS1 cells were replated into culture dishes containing glass coverslips and grown for an additional 24 hr. Coverslips were mounted in complete medium (DMEM with 10% fetal calf serum) and observations were made at ambient temperature by using a Zeiss confocal laser-scanning microscope. To photobleach the P450/GFP chimera in a section of the cell, a narrow strip (1.6 μ m) across the cell was selected and scanned with an argon 488-nm laser at 100% energy until the fluorescence was reduced to the background level. Recovery of the fluorescence was monitored by scanning of the bleached area with the same laser at 10% imaging intensity power until the recovery was complete. Fluorescence images were quantified by using the IMAGEQUANT software program (Molecular Dynamics) and diffusion coefficients, *D*, calculated as described (23).

RESULTS

Cellular Localization of P450/GFP Chimeric Proteins Transiently Transfected in COS1 Cells. The N-terminal 29 amino acids of P450 2C1 have been shown to be sufficient for insertion into the membrane and for retention in the ER of several reporter molecules (14, 15). Likewise, the catalytic domain of P450 2C2 (amino acids 22–490) has been shown to mediate ER retention when fused to the luminal and transmembrane or transmembrane domains of EGFR, a plasma-membrane protein (14). To provide fluorescent tags, GFP was fused to the C terminus of intact P450 or to the catalytic domain of P450 with the EGFR transmembrane domain as a

membrane-insertion sequence. The N-terminal 29 amino acids of P450 2C1 were fused at the beginning of GFP as per previous studies with other reporter proteins (refs. 14 and 15; Fig. 1).

As revealed by fluorescence microscopy (Fig. 2), each protein exhibited a typical ER-like pattern, indicating that the presence of the GFP at their termini did not affect ER retention function. Moreover, both chimeras containing the cytoplasmic domain of P450 2C2, OEC2/GFP, and C2(1–490)/GFP also retain their enzymatic activity. The laurate hydroxylase activities of lysates of transfected COS1 cells were 40 pmol, 44 pmol, and 38 pmol of 11-hydroxylauric acid formed per 100-mm dish for P450 2C2, C2(1–490)/GFP, and OEC2/GFP, respectively. The amounts of immunoprecipitated radiolabeled chimeric proteins were similar to each other and were about half that of P450 2C2 (Fig. 3).

It is possible that the active P450 in the COS1 cells is a minor fraction of the total chimeric protein expressed and is not detectable by examining total fluorescent protein. Expression of P450 protein in transfected COS1 cells is too low for the spectral analysis and quantification of the functional P450 form. However, for the C2(1–490)/GFP chimera, the total amount of GFP protein expressed can be estimated by fluorometry. The specific activity of C2(1–490)/GFP is 0.16 nmol/min per nmol of GFP when the protein is quantified in this way (Table 1). The amount of chimeric P450 expressed in insect cells infected with baculovirus vector is sufficient for detection of protein either by fluorometry or by CO/difference spectra to quantitate the amount of P450. The specific activity of C2(1–490)/GFP calculated on the basis of functional P450 present in whole extracts of insect cells is 0.34 nmol/min per nmol of P450, which is similar to the 0.36 nmol/min per nmol of P450 determined for P450 2C2 in this system (23). The fusion of P450 2C2 to GFP, therefore, does not detectably affect its activity. In the insect cells, the activity of C2(1–490)/GFP is 0.17 nmol/min per nmol of GFP, nearly the same as that determined for the COS1 cells. Therefore, slightly more than 50% of the total C2(1–490)/GFP is functional in the insect system. Assuming that the specific activity, on the basis of functional P450, of C2(1–490)/GFP assayed in COS1-cell lysates is similar to that assayed in insect-cell lysates, then $\approx 50\%$ of the chimera expressed in COS1 cells is also estimated to be active. Because nearly all of the GFP chimera are mobile in the membrane (see below), the finding that a

FIG. 1. Schematic illustration of chimeric proteins structure. Chimera C2(1–490)/GFP contains full-length P450 2C2 (amino acids 1–490) (open box) followed by GFP (black box). Chimera C1(1–29)/GFP contains 29 N-terminal residues of P450 2C1 followed by GFP, whereas chimeras OEC2(22–490)/GFP has the cytoplasmic domain of P450 2C2 (residues 22–490) fused to the GFP and is inserted into the ER membrane by the transmembrane domain of EGFR (hatched box). Chimera EGFR/GFP/C1(1–29) has EGFR signal sequence (shaded box) at the N terminus, followed by the GFP and C-terminally located signal/anchor sequence of the P450 2C1 (amino acids 1–29).

FIG. 2. Fluorescent localization of chimeric proteins expressed in COS1 cells. COS1 cells were transfected with the chimeras shown, and 48 hr later, cells were fixed and observed in the fluorescent microscope with the fluorescein filter and oil objective ($\times 63$).

majority of the chimera is functional indicates that the functional P450 is also mobile.

Mobility of P450/GFP Chimeras in the ER Membranes. The mobilities of the chimeric proteins were examined by using fluorescence photobleaching of transfected cells and measuring the time required for unbleached proteins to move into the bleached area. After photobleaching a narrow (1.6- μ m wide) strip with the laser beam at full power, we monitored the recovery of the fluorescence by imaging the selected area at low energy. Cells were imaged immediately before and after the photobleaching and at 15 s and 45 s of recovery (Fig. 4). Diffusion of all three unbleached chimeras into the bleached

FIG. 3. Immunoprecipitation of radiolabeled proteins expressed in COS1 cells. Forty-eight hours after transfection, COS1 cells were labeled for 4 hr with *trans*- 35 S label, and immunoprecipitated proteins were analyzed by using SDS/PAGE gels. Lane 1 shows proteins from mock-transfected cells, lane 2, proteins from cells transfected with P450 2C2, lane 3, protein from cells transfected with C2(1–490)/GFP, and lane 4, proteins from cells transfected with OEC2/GFP. The arrows indicate the positions of P450 2C2 and its chimeras.

Table 1. Quantification of the C2(1–490)/GFP by fluorometry and determination of P450 specific activity

Cell type	Difference spectra P450 nmols	Fluorescence P450/GFP nmols	Specific activity, nmol/min per nmol of P450	Specific activity, nmol/min per nmol of P450/GFP
Insect	3.33 ± 0.42	6.61 ± 1.3	0.34 ± 0.06	0.17 ± 0.04
COS1	ND	0.017 ± 0.006	ND	0.16 ± 0.03

Chimera C2(1–490)/GFP was expressed in COS1 and insect cells, and the whole-cell extracts were used for the activity assay, difference spectra, and fluorometric measurements. The calculated amounts of the protein are per 100-mm culture dish. The standard error of the mean with *n* = 3 is shown. The activity of purified P450 2C2 is 0.73 nmol/min per nmol of P450 (26). ND, not detectable.

zone was rapid, as shown by nearly full recovery at 45 s. To estimate the mobile fraction of the chimeric proteins, the ratio of fluorescence intensity within and outside the bleached zone before photobleaching and after recovery was compared (21). Between 80% and 90% of all of the chimeras were mobile in the ER membrane. Repeated photobleaching of the same 1.6-μm strip led to the total loss of the fluorescence outside the bleached zone (not shown), which indicates that the proteins diffuse throughout the whole ER and are not restricted to subdomains.

To estimate the mobilities of the proteins in the membrane, a series of photobleaching experiments for each chimera was performed. For each image series, diffusion coefficients (*D*) were calculated as described (25). The diffusion coefficients for all three chimeras were similar, at 5.8×10^{-10} cm²/s for C2(1–490)/GFP, 6.2×10^{-10} cm²/s for C1(1–29)/GFP, and 2.6×10^{-10} cm²/s for OEC2/GFP (Table 2). Although OEC2/GFP consistently showed about one-half the mobility of the C2(1–490)/GFP or chimera C1(1–29)/GFP, the differences were not statistically significant. These results indicate that the P450/GFP chimeras are not immobilized in the ER

membrane; however, their mobility rates are about 10–20% of the rates determined for some Golgi proteins (21). Aggregation of P450 and its chimeras may be responsible for the lower mobility and exclusion from transport vesicles.

If this is the case, the mobility of a protein that is transported from the ER and can presumably enter transport vesicles but is then returned to the ER by a retrieval mechanism should be substantially greater than that of the P450 chimera. Such a protein, with a dilysine ER retention motif, was constructed by substituting Lys at positions –3 and –4 from the C terminus in a chimera of GFP with the N-terminal signal sequence of EFGR at the N terminus for membrane targeting and C1(1–29) at the C terminus as a membrane anchor. In Fig. 5, the ER distribution of this chimera is compared with that of C2(1–490)/GFP and the effects of nocodazole on the distribution of the two proteins are illustrated. Nocodazole-induced disruption of microtubules inhibits retrograde transport of recycled ER proteins, which alters their subcellular distribution (27). Consistent with previous studies, nocodazole treatment did not affect the distribution of C2(1–490)/GFP but rather caused the redistribution of the chimera with the dilysine motif

FIG. 4. Profiles of the fluorescence recovery after photobleaching for the chimera C1(1–29)/GFP (A), OEC2(22–490)/GFP (B), and C2(1–490)/GFP (C). The selected area containing one or few positive cells was imaged immediately before photobleaching (shown as Pre). Line photobleaching was achieved by scanning with 100% laser energy, and fluorescence recovery was monitored by imaging the same area at low energy (10%) at the indicated time points.

Table 2. Diffusion coefficients (*D*) of chimeric proteins retained in the ER membranes

Chimera	<i>D</i> , cm ² /s
C2(1–490)/GFP	$5.8 \pm 0.9 \times 10^{-10}$
C1(1–29)/GFP	$6.2 \pm 0.8 \times 10^{-10}$
OEC2/GFP	$2.6 \pm 0.8 \times 10^{-10}$
EGFR/GFP/C1(1–29)KK	$7.1 \pm 1.1 \times 10^{-10}$

The SDM from *n* = 6 measurements is indicated.

into a punctate pattern that is characteristic of ER proteins that undergo retrieval from the early Golgi compartment (27). The mobility coefficient determined for the chimera EGFR/GFP/C1(1–29)KK was 7.1×10^{-10} cm²/s, which is only slightly greater than the mobilities for the P450 chimeras. This indicates that the aggregation state of this protein is similar to that of the P450 chimera; thus, aggregation per se cannot be responsible for preventing transport of the chimera.

DISCUSSION

These studies indicate that chimeric proteins containing P450 ER retention sequences are not immobilized in the ER membrane. The mobilities measured for the P450 chimeras fall within the range of the mobilities measured for Golgi and plasma membrane proteins. The mobilities are slightly greater than those for plasma membrane proteins and are about 10–20% those of the Golgi proteins (21). They are similar to the mobilities calculated for the model membrane protein, VSVG (Vesicular stomatitis virus 4 protein) (28), with an in-transit mobility from ER to Golgi calculated to be 10×10^{-10} cm²/s (19) and that of human EGFR, calculated to be $0.3\text{--}1.5 \times 10^{-10}$ cm²/s (29). Furthermore, the chimeric proteins are freely mobile throughout the ER, because repeated bleaching of a small region resulted in the loss of fluorescence in the entire ER. The mobility of the chimeric P450 proteins indicates that immobilization by network formation does not prevent the protein from reaching the transport vesicles. Their exclusion from the transport vesicles probably is not explained by the lack of a positive transport signal; some “nonspecific” transport by bulk flow would be expected. It is more likely that either the proteins are incompatible with the formation of a transport vesicle, presumably as a result of the cytoplasmic domain structure, which cannot pack into the protein coat of the vesicle, or they are targeted to areas of the ER membrane

other than those in which transport vesicles are formed. For example, assymetric distribution of lipids and their organization into microdomains (rafts) that may selectively transport proteins has been recently implicated in membrane trafficking (30), and P450 could be concentrated into a microdomain that does not form transport vesicles.

It is possible that P450s form large aggregates which, though mobile, are too large for packaging into a transport vesicle. However, the diffusion coefficients of the P450 chimera were similar to that of a chimeric ER protein that enters transport vesicles and is retained in the ER by recycling. The similar mobilities suggest that these proteins have similar sizes and, thus, similar aggregation states. Therefore, if P450 aggregation prevents transport, the determining factor is the nature, and not just the large size, of the aggregate.

Mobilities were not significantly different for chimeras containing the entire P450 or either the N-terminal signal/anchor sequence or the catalytic domain without the signal/anchor, both of which have been shown to mediate ER retention of heterologous proteins (11, 14, 15). Either of the two redundant retention functions, therefore, could be primarily responsible for the retention. If interaction with other proteins is critical for retention, then either the two sequences can interact with the same proteins or interactions with different sets of proteins by each can result in ER retention. Because the mobilities are similar, complexes that are formed for each must be of comparable size.

Although there are no other studies estimating the lateral mobility in the ER membrane of P450 or other ER resident proteins, Kawato *et al.* (31) have estimated the rotational mobility of P450 by the decay of absorption anisotropy after photolysis of the heme. These studies have led to the conclusions that a significant fraction of microsomal P450 is rotationally immobile and that the fraction of mobile protein depends on the lipid/protein ratio of the vesicles. In addition, it was also shown that drug induction of P450 content further increases the fraction of rotationally immobile P450 in rat-liver microsomes, presumably as a consequence of increased protein–protein interactions (32). These data, as well as studies on the aggregation of partially purified proteins, indicate that P450 forms complexes with other proteins in the ER membrane that reduce the rotational mobility. In the rotational-diffusion studies, molecules with rotational frequencies of greater than a few milliseconds are considered immobile (31). Because essentially all of the P450 chimeras are mobile laterally when observed on a ≈ 1 -second time scale, transient interactions of P450 with other molecules probably underly the rotational immobility. About 50% of the molecules are rotationally immobile so that the average lateral mobility may be expected to be reduced by about one-half by the transient interactions.

The diffusion coefficient of Golgi-specific membrane proteins, mannosidase II and galactosyltransferase, fused to GFP has been estimated to be 3×10^{-9} cm²/s and 5×10^{-9} cm²/s, respectively (21). The mobilities estimated for these Golgi proteins were about 10–30 times higher than the mobilities calculated for some plasma-membrane proteins (18, 33) and about 5- to 8-fold higher than those measured for the P450 chimeras. The diffusion of plasma-membrane proteins may be restricted by both cytoplasmic and extracellular barriers (18, 33). The difference between the P450 ER proteins and the Golgi proteins may be related partly to differences between the Golgi and ER membranes, because the mobility of Golgi proteins redistributed to the ER by brefeldin treatment was one-half of that in the Golgi (21), although mobility within ER membranes for the nuclear-membrane protein, lamin B receptor was similar to that of the Golgi proteins (25). Alternatively, as noted above, the decreased mobility of the P450 chimeras in the ER may reflect incorporation into larger complexes compared with the Golgi proteins. Diffusion coef-

FIG. 5. Effect of nocodazole on the subcellular distribution of chimeras C2(1–490)/GFP and EGFR/GFP/C1(1–29)KK. COS1 cells were transfected with C2(1–490)/GFP (A and C) or EGFR/GFP/C1(1–29)KK (B and D), and 48 hr later were incubated for 2 hr in the presence or absence (Control) of 10 μ M nocodazole. Fixed cells were observed and photographed in the fluorescent microscope with the fluorescein filter and oil objective ($\times 63$).

ficients for ER-membrane resident proteins other than cytochrome P450 have not been estimated, so studies of additional proteins will be needed to determine whether the differences observed between the P450 chimeras and Golgi proteins are specific to the proteins or to the organelle membrane.

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