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Fluorescence Resonance Energy Transfer Studies on the Interaction between the Lactate Transporter MCT1 and CD147 Provide Information on the Topology and Stoichiometry of the Complex *in Situ**

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The monocarboxylate (lactate) transporters MCT1 and MCT4 require the membrane-spanning glycoprotein CD147 for their correct plasma membrane expression and function. We have successfully expressed CD147 and MCT1 tagged on their C or N termini with either the cyan (CFP) or yellow (YFP) variants of green fluorescent protein. The tagged proteins were correctly targeted to the plasma membrane of COS-7 cells and were functionally active. Measurements of fluorescence resonance energy transfer (FRET) between all combinations of the tagged proteins were made. FRET was observed when either the C or N terminus of MCT1 (intracellular) is tagged with CFP or YFP and co-expressed with CD147 tagged with YFP or CFP on the C terminus (intracellular) but not the N terminus (extracellular). FRET was also observed between two CD147 molecules when both YFP and CFP were on the C terminus but not when both were on the N terminus or one on either end. No FRET was observed between MCT1-YFP and MCT-CFP in any combination. A wide range of controls including photobleaching were employed to confirm that where FRET was observed, it was not an artifact of direct excitation of YFP by the CFP excitation laser. It was also shown that nonspecific overcrowding of proteins did not induce FRET. Because FRET only occurs between two fluorophores if they are less than 100 Å apart and in a suitable orientation, our data provide important information on the topology of CD147 and MCT1 within the plasma membrane. The minimum configuration consistent with the data is a dimer of CD147 associating with two MCT1 molecules such that the C terminus of CD147 in the cytosol is close to the C terminus of its partner CD147 and to the C and N termini of an associated MCT1 molecule. FRET may provide a noninvasive technique for measuring changes in these interactions in living cells.

The transport of lactic acid across the plasma membrane is of critical importance to all cells. Thus for glycolytic cells such as white muscle fibers, glia cells, most tumor cells, and blood cells, as well as all other cells under hypoxic conditions, two lactic acids must exit the cell for each glucose utilized. In other tissues, lactic acid is transported into the cell for gluconeogenesis and lipogenesis (liver, kidney) or oxidation as a major respiratory fuel (heart, skeletal muscle, and brain) (1). Transport is mediated by a family of proton-linked MonoCarboxylate Transporters (MCTs)[1] of which nine members have been identified (2), although only for MCTs 1–4 has transport of lactic acid been demonstrated directly (3–5). Recently a new member of the MCT family has been identified with only 20–30% identity to MCT1. This acts as a sodium-independent aromatic amino acid transporter (6), suggesting that MCT family members may have a wider role.

In the red blood cell, 4,4′-diisothiocyanostilbene-2,2′-disulfonate was found to cross-link MCT1 to GP70 (Embigen) (7, 8), a cell surface glycoprotein that is a member of a small family of closely related proteins (9), including CD147 (also known as OX-47, EMMPRIN, HT7, and basigin) and SDR1 (10). These are predicted to have common structural features including immunoglobulin-like domains in the extracellular region, a short C-terminal cytoplasmic tail, and a single transmembrane domain (11). Although GP70 is not widely distributed, CD147 is (12–15). This led us to consider whether GP70 and CD147 might be required for the proper expression of MCTs. Consistent with this, we have shown that antibodies against CD-147 co-immunoprecipitated MCT1 and MCT4 but not MCT2. The interaction between CD147 and MCT1 or MCT4 was confirmed in cultured cell lines by using cross-linking antibodies against CD147 to redistribute the CD147 to one end of the cell as a cap. MCT1 and MCT4 were found to redistribute into the caps with CD147, whereas GLUT1 and MCT2 did not (16). In addition we have demonstrated that in all tissues expressing MCT1 or MCT4, CD147 is always expressed in exactly the same location (16). These observations all imply a close association between CD147 and MCT1/MCT4 within the plasma membrane, but our data also suggested that this interaction is critical for the correct expression of MCT1 and MCT4 at the cell surface. Thus when CD147 was co-expressed with MCT1 or MCT4 in mammalian cell lines, both proteins were correctly targeted to the plasma membrane, and lactate transport activity was greatly increased. In contrast, when MCT1 or MCT4 was expressed in

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[1] The abbreviations used are: MCT, monocarboxylate transporter; BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; CFP, cyan fluorescent protein; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; YFP, yellow fluorescent protein; PCR, polymerase chain reaction.

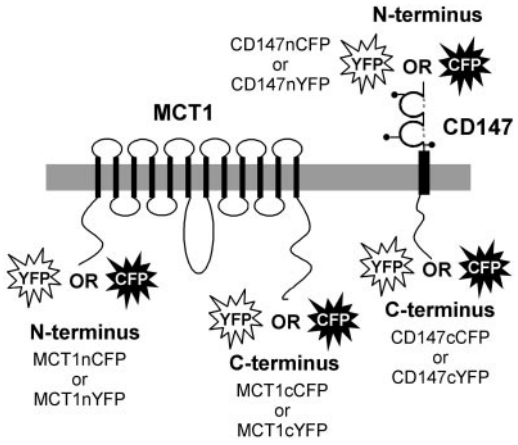

FIG. 1. **Scheme illustrating the different CFP and YFP constructs used and their nomenclature.**

the absence of CD147, the proteins accumulated in the endoplasmic reticulum/Golgi apparatus rather than being expressed in active form in the plasma membrane (16). Furthermore, we have shown that co-injection of *Xenopus* oocytes with MCT1 cRNA and an antisense cRNA to a *Xenopus* homologue of CD147 reduced MCT1 expression and activity (17). These data imply that CD147 acts as a chaperone for MCT1 and MCT4 translocation to the plasma membrane.

To study such interactions in a living cell in real time a non-invasive technique is required. In this paper we describe how this can be achieved using fluorescence resonance energy transfer (FRET) between the two proteins tagged on their C or N termini with either the cyan (CFP) or yellow (YFP) variants of GFP. FRET between two fluorophores only occurs if they are less than 100 Å apart and in the right orientation. As such, it is able to provide valuable information about protein/protein interactions and associated conformational changes that may occur (18, 19). FRET has been used extensively to study interactions between soluble proteins or a membrane protein and a soluble protein binding partner (6, 20–23), but very few studies have successfully employed FRET to study the interaction of membrane proteins in their native environment (24–26). To our knowledge this is the first report describing the use of FRET to detect an interaction between two distinct proteins that functionally associate in the plasma membrane, although oligomerization of the human serotonin transporter and the rat γ-aminobutyric acid transporter has been detected in this way (26).

EXPERIMENTAL PROCEDURES

Production of MCT1 and CD147 Tagged with GFP, CFP, and YFP—We placed both MCT1 and CD147 in CFP and YFP expression vectors (CLONTECH) that allowed expression of the C- or N-terminally CFP- and YFP-tagged proteins, respectively; *i.e.* a total of eight constructs in all. The nomenclature used for these constructs is illustrated in Fig. 1. In outline, MCT1nCFP or MCT1nYFP represents the N-terminal CFP- and YFP-tagged proteins, and MCT1cCFP and MCT1cYFP the corresponding C-terminal tagged proteins. CD147nCFP, CD147nYFP, CD147cCFP, and CD147cYFP represent the equivalent CD147 constructs. For MCT1cGFP, MCT1cCFP, and MCT1cYFP, the coding region of rat MCT1, previously cloned into the pCIneo mammalian expression vector (16), was amplified by PCR using primers modified by the addition of an *Eco*RI site at the 5′ end and removal of the stop codon plus addition of a *Sal*I site at the 3′ end. Following PCR, fragments were digested with *Eco*RI and *Sal*I and cloned in the correct reading frame into the corresponding sites of the pEGFP-N1, pECFP-N1, and pEYFP-N1 vectors (CLONTECH). A similar procedure was used for CD147cCFP and CD147cYFP, but the 5′ and 3′ restriction sequences used were both *Eco*RI. For MCT1nCFP and MCT1nYFP, MCT1 was subcloned from pCIneo-MCT1 by cutting out the coding region with *Eco*RI and inserting in frame into the pECFP-C1 and pEYFP-C1 vectors (CLONTECH). CD147nCFP and CD147nYFP were produced by PCR with primers that inserted *Eco*RI and *Bam*HI sites at the 5′ and 3′ ends, respectively. Following PCR, fragments were digested with *Eco*RI and *Bam*HI and cloned in the correct reading frame into the corresponding sites of the pECFP-C1 and pEYFP-C1 vectors. The resulting constructs were checked by sequencing and expressed in COS cells by a liposome-mediated transfection procedure as detailed below.

Transfection of COS-7 Cells—COS cells were maintained in Dulbecco's modified Eagle's medium (from Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine (Invitrogen), 100 units/ml penicillin, and 100 μg/ml streptomycin (Sigma). For FRET studies, coverslips were placed in 6-well tissue culture plates and seeded with 1–3 × 10^5 cells per well in 2 ml of complete medium, whereas for studies of lactate transport, 10-cm plates were seeded with 2–5 × 10^5 cells in 10 ml of complete medium. Cells were incubated at 37 °C in a CO_2 incubator until they were 60–70% confluent, and then transfection was carried out essentially as described previously (16). DNA (2 μg) was mixed with 6 μl of FuGENE-6 (Roche Molecular Biochemicals) in 100 μl of serum-free medium and incubated for 20 min before being added to the cells and incubating for 24 h. At this point the medium was replaced, and live imaging was performed after an additional 24 h.

Live Imaging of Cells and FRET—Cells were imaged at 37 °C in imaging buffer, 136 mM NaCl, 4.7 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, 2 mM $NaHCO_3$, 5 mM sodium phosphate, 25 mM Hepes pH 7.4, using one of two systems. The majority of experiments was performed with a Leica confocal imaging spectrophotometer system (TCS-SP2) attached to a Leica DMIRBE inverted epifluorescence microscope and equipped with an argon laser (458-, 476-, 488-, 514-nm lines) and acousto-optic tunable filter to attenuate individual visible laser lines. For some photobleaching studies a Leica DMIRBE inverted epifluorescence microscope was employed with conventional excitation through specific filters for CFP, GFP, YFP, and image analysis was performed using Improvision OpenLab software. In both cases a 63 × 1.32 NA oil immersion objective was employed. Quantification of FRET was determined by measurement of the ratio of intensity of the fluorescence emission signal at 480 nm to 530 nm when excited at the CFP excitation wavelength (458 nm). The potential artifacts that may distort this value and the means of overcoming them are discussed in detail under "Results."

Lactate Transport into Transfected COS Cells and Xenopus Oocytes—Initial rates of L-lactate transport into control and transfected COS cells were determined at 25 °C from the rate of intracellular acidification measured using the intracellular pH-sensitive fluorescent dye 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) as previously described (16). Excitation was at 440 and 490 nm, emission was at >530 nm, and intracellular acidification was detected as an increase in the 440 nm/490 nm fluorescence ratio. The initial rate of this fluorescence ratio change ($\Delta F \cdot s^{-1}$) is directly proportional to the rate of substrate-mediated proton transport expressed in nanomoles of protons per μl intracellular volume and was determined by fitting the data to a first order rate equation as described previously (27). For *Xenopus* oocytes, transfection with MCT1cGFP was performed by microinjection of the cRNA synthesized by *in vitro* transcription, and initial rates of lactate transport were determined at 20 °C using BCECF fluorescence as described previously (5).

RESULTS

MCT1-GFP Is an Active Lactate Transporter—To confirm that attachment of a GFP to MCT1 does not alter the ability of the latter to act as a lactate transporter, we first expressed MCT1 with a C-terminal GFP in *Xenopus* oocytes. Transport activity was determined from the rate of decrease in intracellular pH (pH_i) that accompanies proton-linked lactate entry, using BCECF as described under "Experimental Procedures." Data are shown in Fig. 2 and confirm that the MCT1-GFP construct is transporting lactate in a similar fashion to native MCT1 but with a slightly reduced affinity. Thus the K_m value (± S.E.) of MCT1-GFP was 11 ± 2.1 mM compared with a value of 4.4 ± 0.7 mM for normal MCT1 (5).

Expression of CFP- and YFP-tagged MCT1 and CD147 in COS Cells—Each one of the eight constructs was successfully expressed in COS cells and could be visualized in live cells by confocal microscopy. Some examples are illustrated in Figs. 3–5. However, when expressed alone these constructs did not show a distinct membrane plasma membrane distribution but

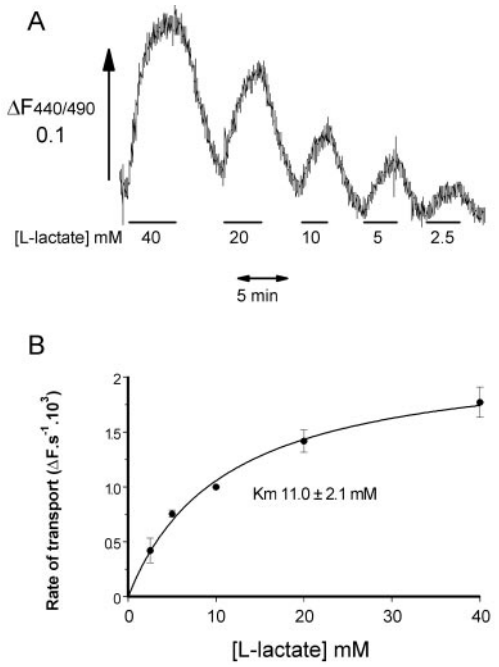

FIG. 2. **Characterization of the kinetics of MCT1cGFP in *Xenopus* oocytes.** Oocytes were microinjected with cRNA for MCT1 tagged with C-terminal GFP, and after 3 days, L-lactate transport studies were performed using BCECF fluorescence as described previously (5). *A* presents crude data for a single oocyte at the L-lactate concentrations shown. Note that water-injected eggs show no measurable L-lactate transport by this technique. *B* represents mean data (± S.E., *error bars*) for initial rates of transport into four separate oocytes determined by first order regression of the fluorescence trace. Data were then fitted to the Michaelis-Menten equation by least squares regression analysis to obtain the K_m value.

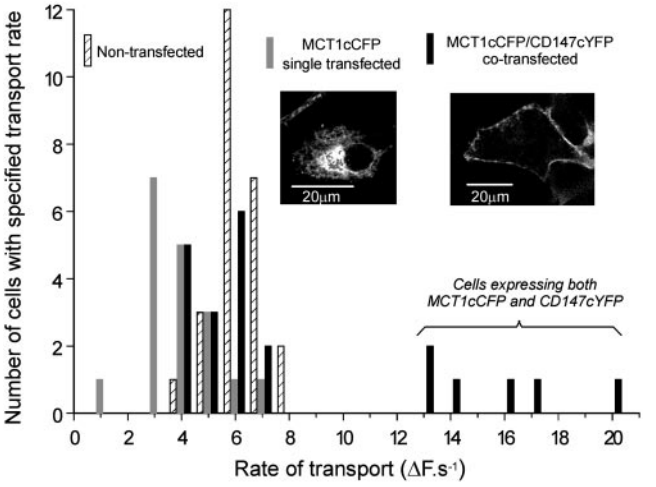

FIG. 3. **COS cells transfected with both MCT1nCFP and CD147cYFP show enhanced lactate transport activity.** COS-7 cells were transiently transfected with either MCT1cCFP alone or together with CD147cYFP. The two images shown were visualized by live imaging using a confocal microscope and are representative of the transfected cell present (40–50% for MCT1cCFP alone and about 20% for dual transfection). Initial rates of L-lactate (20 mM) transport into a field of cells were determined using BCECF fluorescence as described under "Experimental Procedures," and the number of cells with each rate of transport was counted. Data are presented for non-transfected cells (*hatched bars*), single transfection with MCT1cCFP alone (*gray shaded bars*), and dual transfected with MCT1cCFP and CD147cYFP (*solid bars*). Only with dual transfection did any cells (about 20%) exhibit enhanced (3-fold) rates of transport, and these are taken to represent cells expressing both constructs.

rather appeared to be associated with the perinuclear region (Golgi plus endoplasmic reticulum). This was particularly the case for MCT1 in agreement with our previous observations with untagged MCT1 (16). Examples can be seen for MCT1cCFP and MCT1nCFP in Figs. 3 and 4*A*, respectively. In contrast, when both an MCT1 and a CD147 construct were co-expressed, both proteins displayed a plasma membrane location as illustrated in Figs. 3 and 4, *C* and *D*. Once again this is the same as observed for the untagged proteins (16) and confirms that the CFP and YFP attached to the C and N termini do not interfere with the correct targeting of the proteins.

To confirm that lactate transport was enhanced in those cells expressing both proteins, rates of transport were determined in a large number of individual cells using BCECF fluorescence. This was carried out with control cells or cells transfected with either MCT1cCFP alone or together with CD147cYFP. Fluorescence confocal microscopy revealed that such transient transfection only induced expression of the tagged proteins in a fraction of the cells; about 40–50% in singly transfected and 20–30% in dual transfected cells. Thus it was important to distinguish between non-transfected and transfected cells when analyzing rates of transport by individual cells. Unfortunately, the sensitivity of the imaging system used for transport measurements was not sufficient to determine this directly from the CFP and YFP fluorescence. However, when rates of transport were determined it was clear that in cells transfected with both MCT1cYFP and CD147cCFP, about 25% exhibited greatly enhanced rates of transport as shown in Fig. 3. This is consistent with these cells expressing both MCT1cYFP and CD147cCFP in an active form.

FRET Occurs between CFP- and YFP-tagged CD147 and MCT1 Only When Both Tags Are Intracellular—The emission spectra of CFP-tagged and YFP-tagged proteins were first determined in cells transfected with a single construct and excited with the laser lines appropriate for CFP and YFP (458 and 514 nm, respectively). Data for MCT1nCFP and CD147cYFP are illustrated in Fig. 4, *A* and *B*, respectively, and show that the two spectra are quite distinct with emission peaks at about 480 and 530 nm, respectively. For the measurement of FRET, cells expressing both CFP and YFP constructs were excited at 458 nm. If FRET occurs, a shift in the emission spectrum of the CFP construct should be observed as direct energy transfer occurs from the excited CFP to the YFP with subsequent emission of the fluorescent light at 530 nm rather than 480 nm. In cells expressing both MCT1nCFP and CD147cYFP, this was observed as a decrease in the 480 nm emission and an increase in the 530 nm emission compared with that seen with the CFP construct alone as shown in Fig. 4*C*. Thus the ratio of 480 nm to 530 nm emission intensity acts as an indicator of the extent of FRET. However, it is not possible to quantify the extent of FRET from this ratio alone because YFP is also excited by 458 nm light at a level about 30% of that induced at 514 nm. Such "bleed-through" will cause the absolute value of the ratio to be less than 2.5 even in the absence of FRET, and the magnitude of this decrease will be dependent on the expression level of the YFP construct relative to the CFP construct. Thus, if the expression of the YFP protein were to be greatly in excess of the CFP protein, discriminating between such bleed-through and real FRET would present a serious problem. For this reason conditions were optimized to ensure that expression of the YFP construct was less than or equal to that of the CFP construct. This was most readily achieved by using MCT1 constructs tagged with CFP and CD147 constructs tagged with YFP, although for completion all combinations were employed.

In Table I we present summary data for the 480 nm:530 nm emission intensity ratio for all combinations of CFP and YFP constructs. The shaded boxes highlight situations in which

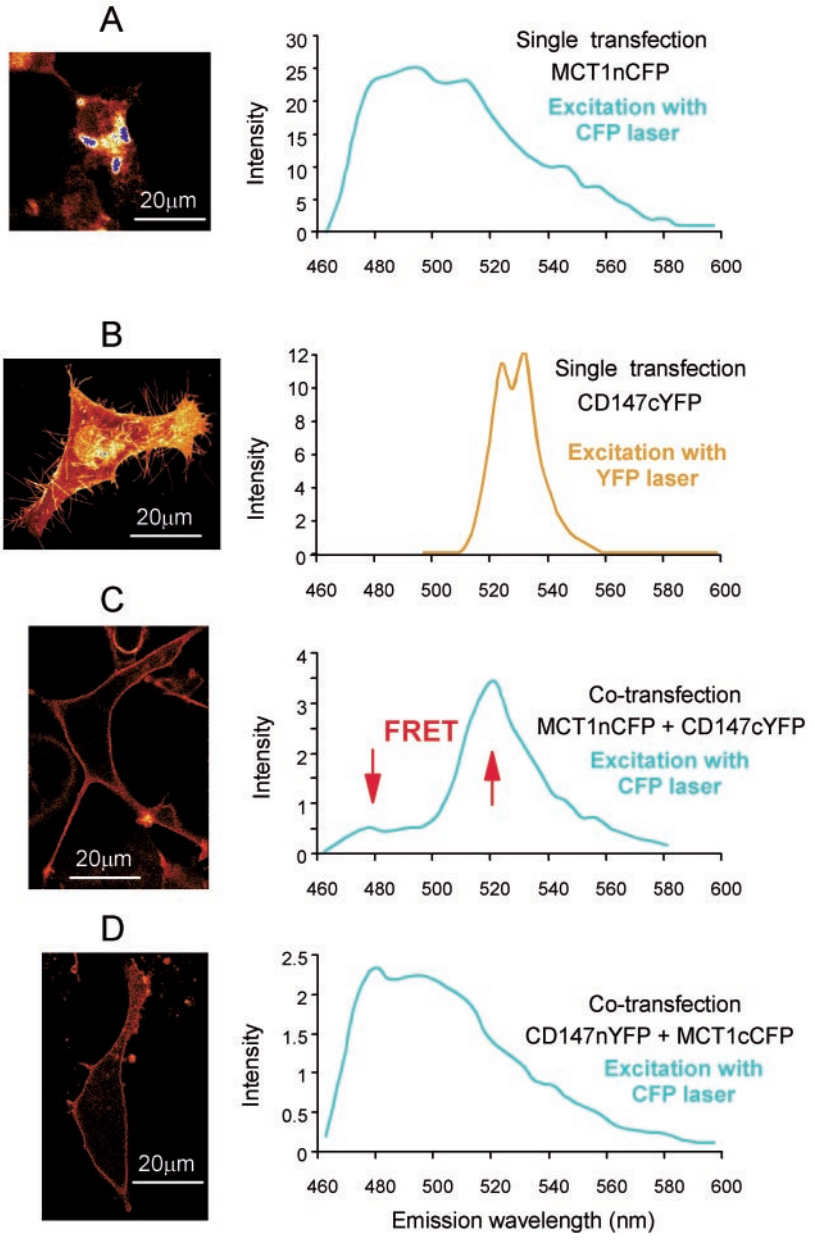

FIG. 4. **Fluorescence emission spectra of COS-7 cells expressing MCT1nCFP, CD147cYFP, and CD147nYFP alone or in combination.** Transient transfection with the relevant constructs was performed as described under "Experimental Procedures." Cells were imaged using the Leica confocal imaging spectrophotometer system (TCS-SP2) using the 458 nm and 514 nm lines of the laser to excite CFP and YFP, respectively. In each case, the intensity of the fluoresced light for the cell shown was determined over the range of wavelengths indicated. In the cells co-transfected with MCT1nCFP and CD147cYFP (fluorophores both intracellular) the decrease in 480 nm emission and increase in 530 nm emission implies that FRET is occurring (*C*). This was not observed when cells were co-transfected with MCT1cCFP and CD147nYFP (fluorophores intracellular and extracellular, respectively) as shown in *D*.

the ratio is especially low, with values (± S.E. of (*n*) observations) of 0.39 ± 0.03 (6) for CD147cYFP with MCT1nCFP, 0.28 ± 0.09 (6) for CD147cCFP with MCT1nYFP, 0.30 ± 0.10 (5) for CD147cCFP with MCT1cYFP, and 0.41 ± 0.05 (5) for CD147cYFP with MCT1cCFP. Because the C terminus of CD147 and both the C and N terminus of MCT1 are intracellular, these all represent situations in which both fluorescent tags are intracellular. This is consistent with FRET occurring as a result of the close proximity of the C terminus of CD147 to both the C and N termini of MCT1. Furthermore, the low values are independent of whether the CFP and YFP are on CD147 or MCT1 as would be predicted for FRET. In contrast, much higher ratios were obtained when CD147nYFP was co-expressed with MCT1cCFP (Fig. 4*D*) or when other combinations of CD147 tagged with either CFP or YFP and either C- or N-terminally tagged MCT1 were co-expressed (Table I). These higher values are considered to represent situations in which FRET is not occurring and act as important negative controls. This is especially true when CD147 has its CFP or YFP attached to the N terminus that is extracellular and thus distant from either the C or N terminus of MCT1 in the cytosol. FRET is dependent on the two fluorophores being within 50–100 Å of each other (18, 19), and this will not be possible under such conditions.

Photobleaching Experiments Confirm the FRET Measurements—To be more certain that the evidence for FRET described above is valid, we have employed photobleaching of the YFP using a high intensity exposure to the YFP excitation light. When CFP and YFP are exhibiting FRET, photobleaching of the YFP should lead to an increase in the 480 nm emission of the CFP protein. In Fig. 5 we illustrate that this is indeed the case. Two different fluorescence microscopes were used for these experiments. In *panel A*, a conventional fluorescence microscope was used in which control of the excitation wavelength could be optimized for the CFP and YFP excitation maxima using filters of 440 ± 21 nm and 500 ± 25 nm, respectively. The lower wavelength of the CFP excitation light minimizes bleed-through causing YFP excitation; this was not possible with the laser confocal microscope that was constrained by the available laser line (458 nm). It can be seen that following bleaching of the CD147cYFP, the 480 nm emission signal of the MCT1nCFP actually increased. If bleed-through were re-

TABLE I
FRET measurements for all CFP and YFP-tagged constructs of MCT1 and CD147

Cells were transiently co-transfected with the combinations of CFP- and YFP-tagged MCT1 and CD147 shown, and the intensity ratio for emission at 480 and 530 nm was determined for excitation with the 458-nm laser line. In co-transfections such as MCT1nCFP with MCT1nYFP or CD147nCFP with CD147nYFP, untagged MCT1 or CD147 was also transfected to ensure correct plasma membrane targeting of the tagged proteins. Indeed, cells were only analyzed when correct targeting of both tagged proteins to the plasma membrane was observed, because this was also a good indicator that both proteins were expressed at similar levels. Full emission spectra were obtained with excitation at 458 nm, and the intensity ratio for emission at 480 and 530 nm was calculated. Data are expressed as means ± S.E. of the number of observations shown, and the arrangement of the fluorophores is indicated schematically underneath to aid interpretation of the data. FRET is indicated by the *shaded boxes*.

	MCT1cYFP	MCT1nYFP	CD147cYFP	CD147nYFP
MCT1cCFP	0.66 ± 0.02 (24)	0.73 ± 0.05 (13)	**0.41 ± 0.05 (5)**	1.26 ± 0.12 (14)
MCT1nCFP	0.98 ± 0.03 (5)	0.74 ± 0.07 (10)	**0.39 ± 0.03 (6)**	1.33 ± 0.07 (7)
CD147cCFP	**0.30 ± 0.10 (6)**	**0.28 ± 0.09 (6)**	**0.39 ± 0.04 (18)**	0.96 ± 0.05 (5)
CD147nCFP	0.74 ± 0.02 (4)	0.72 ± 0.06 (4)	0.71 ± 0.04 (2)	0.72 ± 0.04 (8)

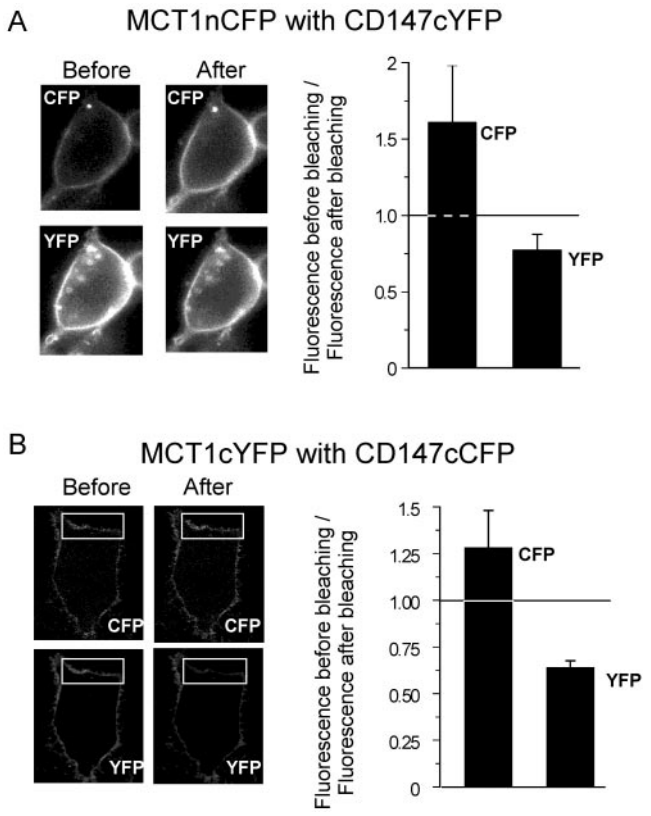

FIG. 5. **The use of photobleaching to confirm that FRET is occurring.** Cells were co-transfected with either MCT1nCFP and CD147cYFP (*A*) or MCT1nYFP and CD147cCFP (*B*) and imaged using either the Leica TCS-SP2 confocal microscope with excitation using the 458 and 514 nm laser lines as in Fig. 4 (*B*) or the Leica DMIRBE inverted epifluorescence microscope with excitation at 440 ± 20 nm and 500 ± 25 nm (*A*). Imaging was performed before and after bleaching the YFP by exposure to the 514 nm laser line for 20 s (*B*) or the 500 nm excitation light for 30 s (*A*). Images are shown for both the CFP and YFP emission (480 and 530 nm, respectively) together with *bar graphs* of the ratio of the emission intensity before bleaching to that after bleaching. These data are given as means ± S.E. for 3 (*A*) or 10 (*B*) different cells. For *A*, ratios are for the whole cell fluorescence, whereas for *B* only a small portion of the plasma membrane was analyzed as indicated on the image.

sponsible for the apparent FRET it would be expected that the 480 nm emission signal would decrease in similar fashion to the 530 nm signal. Similar results were obtained when the experiments were performed with the laser confocal microscope (*panel B*). In this case the fluorescence signal from a small portion of the plasma membrane was analyzed to ensure that only properly targeted proteins were included.

FRET Measurements Reveal Interactions between Two CD147 Molecules but Not between Two MCT1 Molecules—We have also investigated whether FRET occurs between CFP-CD147 and YFP-CD147 or between CFP-MCT1 and YFP-MCT1 when these proteins are expressed together. For this purpose it was also necessary to co-express untagged CD147 or MCT1 with the two tagged MCT1 or CD147 constructs to ensure correct translocation of all proteins to the plasma membrane. Data are shown for all combinations in Table I as 480 nm:530 nm fluorescence emission ratios. These data imply that FRET occurs between CD147cYFP and CD147cCFP (0.39 ± 0.04) but not between either CD147nCFP and CD147nYFP (0.72 ± 0.04), CD147nYFP and CD147cCFP (0.96 ± 0.05), or CD147nCFP and CD147cYFP (0.71 ± 0.04). For MCT1 there was no evidence for FRET between any combination of CFP and YFP constructs.

FRET Is Not the Result of Nonspecific Protein Interactions Due to Overcrowding—It could be argued that the FRET signal was occurring as a result of the close proximity of proteins that are crowded together as a result of overexpression. Although this is unlikely in view of the negative controls described above, the possibility was further eliminated by co-expressing CFP and YFP constructs of CD2 and MCT1. CD2 is another single transmembrane-spanning member of the immunoglobulin superfamily that we have shown previously is unable to substitute for CD147 as a chaperone for correct MCT1 expression (16). We found that the most suitable levels of expression were obtained using the combination of CD2cCFP with MCT1nYFP, and data for these are shown in Fig. 6. It is clear that there is no evidence for FRET under these conditions, despite both proteins being well expressed.

DISCUSSION

It is becoming apparent that many transporters require ancillary proteins for their expression and correct function. Thus CD147 is associated with the lactate transporters MCT1 and MCT4 and is required for their correct expression on the cell surface (16), whereas the 4F2 heavy chain (CD98) interacts with and regulates expression of a family of amino acid transporters (28–31). Similarly, the translocation of the AE1 anion exchanger (Band 3) to the plasma membrane of *Xenopus* oocytes is greatly enhanced by co-expression of glycophorin, which may also modify the activity of AE1 (32). Furthermore, the single-pass glycoprotein CD36 and a 12-pass protein have been implicated in long-chain fatty acid transport across the plasma membrane (33). Membrane receptors and ion channels have also been reported to associate with ancillary proteins that can modulate their activities (34, 35). To study these interactions in living cells a non-invasive technique is required,

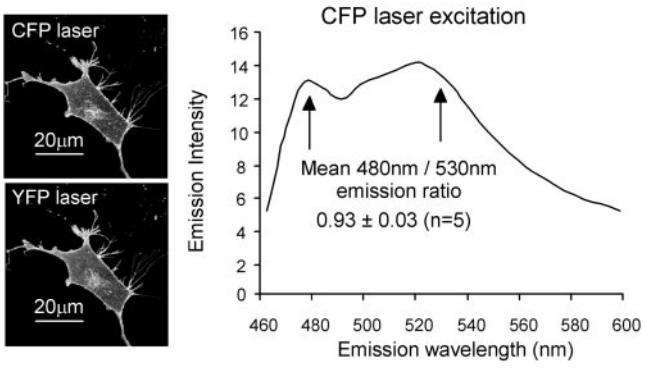

FIG. 6. **Cells co-expressing CD2cCFP with MCT1nYFP do not exhibit FRET.** COS cells were transiently transfected with CD2cCFP with MCT1nYFP and imaged as described in Fig. 4. The emission spectra of the cell shown excited with the 458 laser line is presented and provides no evidence of FRET as confirmed by the mean 480 nm/530 nm emission ratio of 0.93 ± 0.03 for five such cells.

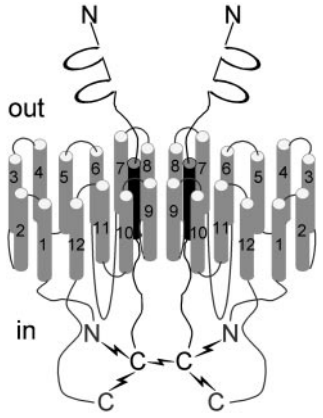

FIG. 7. **Hypothetical model of the topology of CD147 and MCT1 in the plasma membrane.** Details of the rationale behind the dimeric model shown are described in the text. The arrangement of the 12 transmembrane α helices should only be considered as illustrative because it is based on that of the Na^+/H^+ antiporter of *E. coli*, the three-dimensional structure of which has been solved (36). The proximity of N and C termini of MCT1 to the C terminus of CD147 will enable FRET between CFP and FYP tags, as will be the case between CFP and YFP tags on the C termini of two CD147 molecules.

and in this paper we describe how this can be achieved using FRET between CFP and YFP constructs of MCT1 and CD147.

Our data demonstrate that FRET is observed when either the C or N terminus of MCT1 is tagged with CFP and YFP and co-expressed with CD147 tagged with YFP or CFP on the C terminus but not the N terminus. We have performed a wide range of controls to confirm the validity of the FRET data and to exclude the possibility that our data are an artifact of bleed-through of the CFP laser excitation to give direct excitation of YFP. These controls included the use of different CFP/YFP combinations, which exhibited different levels of expression and also YFP photobleaching that induced an enhancement of the CFP fluorescence. The possibility that FRET was the result of nonspecific interactions between proteins caused by over-crowding was rendered unlikely by the inability of CD2cCFP to exhibit FRET with MCT1nYFP. Furthermore, no FRET was observed when MCT1cCFP and MCT1cYFP were co-expressed together with CD147 to ensure that both the tagged proteins were properly targeted to the plasma membrane (Table I). In the absence of CD147 both the CFP and YFP-MCT1 accumulated in the perinuclear region (Figs. 3 and 4), but again there was no evidence of FRET (data not shown). In the light of these controls we conclude that CD147 and MCT1 interact in such a way that CFP or YFP on either the C or N terminus of MCT1 can exhibit FRET with a fluorescent partner on the C terminus of CD147 but not its N terminus. This would indicate that the intracellular C terminus of CD147 is within 50–100 Å of the C and N termini of MCT1, both of which are also cytosolic. In contrast, as would be predicted, the N terminus of CD147, which is extracellular, does not interact in this way. Our data also allow us to draw conclusions about how two MCT1 molecules or two CD147 molecules interact with each other. Co-expression of CFP and YFP constructs of CD147 demonstrate that two CD147 molecules must be in close proximity such that their C termini but not their N termini interact with each other. These data would imply that CD147 is present in the membrane as a dimer (or higher oligomer). In contrast no such interactions are observed between any combination of CFP-MCT1 and YFP-MCT1 implying that their C and N termini are likely to be some distance apart.

Fig. 7 presents a model that is consistent with our data of how MCT1 and CD147 may interact in the plasma membrane. The arrangement of the 12 transmembrane α helices should only be considered as illustrative because it is based on that of the Na^+/H^+ antiporter of *Escherichia coli* whose three-dimensional structure has been solved (36). We suggest that each MCT1 interacts with a single CD147 that in turn forms a dimer with another CD147/MCT1 pair. Such a model would explain how cross-linking antibodies against CD147 cause both the CD147 and MCT1 to aggregate together as a single cap at one end of the cell (16), which would not be possible if the MCT1 interacted with a single CD147. CD147 and its homologues such as GP-70 possess immunoglobulin-like domains in the extracellular region, a short cytoplasmic tail, and a single transmembrane domain (11). The transmembrane region shows a very high degree of cross-species conservation (15) including a centrally positioned glutamic acid residue. A charged amino acid deep within the transmembrane region is a common feature of proteins with multiple transmembrane domains (38) but unusual for a protein with a single transmembrane domain (39), implying an important function. Thus, we have argued previously (16) that critical for the association of the two proteins is an interaction between this transmembrane domain of CD147 and a transmembrane domain of MCT1. This might be helix 8 that contains a conserved arginine known to be essential for normal transporter activity (37). Indeed we have created a glutamate to arginine mutant of CD147cYFP and found that this neither reaches the plasma membrane itself nor enables MCT1 to be expressed at the plasma membrane (data not shown). The model of Fig. 7 assumes that the transmembrane domain of CD147 and helix 8 of MCT1 are closely associated. This could allow the C terminus of CD147 (40 amino acids) and the N or C terminus of MCT1 (15 and 57 residues, respectively) to be close enough for FRET to occur between the attached CFP and YFP moieties.

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Fluorescence Resonance Energy Transfer Studies on the Interaction between the Lactate Transporter MCT1 and CD147 Provide Information on the Topology and Stoichiometry of the Complex *in Situ*

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