In vitro study of placental trophoblast calcium uptake using JEG-3 human choriocarcinoma cells

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Summary

During human fetal development, placental syncytiotrophoblasts actively transport calcium from the maternal to the fetal circulation. Two functional components, a cytosolic Ca2+-binding protein (CaBP) and a Ca2+-ATPase have been identified in the syncytiotrophoblasts of the chorionic villi. We report here the calcium uptake properties of a human choriocarcinoma cell line, JEG-3, which was used as an in vitro model cell system for the syncytiotrophoblasts. In culture, JEG-3 proliferated as large syncytial aggregates expressing typical syncytiotrophoblast markers. 48 Ca uptake by JEG-3 was a substrate- and temperature-dependent, membrane-mediated active process that exhibited linear kinetics for up to 7 min. Both the CaBP and the Ca2+-ATPase were expressed by JEG-3, on the basis of biochemical, histochemical, immunochemical and/or mRNA assays. Immunohistochemistry and in situ hybridization revealed that JEG-3 cells were heterogeneous with respect to the expression of the CaBP. The Ca2+-ATPase activity of JEG-3 was similar to the placental enzyme in terms of sensitivity to specific inhibitors, and was detected histochemically along the cell membrane. Fura-2 Ca2+ imaging revealed that calcium uptake by JEG-3 was not accompanied by a concomitant increase in cytosolic [Ca2+], suggesting a specific Ca2+ sequestration mechanism. The involvement of calciotropic hormonal regulation was evaluated by studying the response of JEG-3 to 1,25-dihydroxy vitamin D3. Calcium uptake was significantly stimulated in a dose-dependent manner by a 24-h treatment of the cells with 1,25-dihydroxy vitamin D3 (optimal dose ~0.5 nM): the CaBP level doubled whereas steady-state CaBP mRNA did not, suggesting that CaBP expression was regulated by 1,25-dihydroxy vitamin D3. These observations strongly suggest that the JEG-3 human choriocarcinoma cells should serve as a convenient in vitro model system for studying the cellular mechanism and regulation of transplacental calcium transport.

Key words: calcium-binding protein, Ca2+-ATPase, membrane transport, placental calcium transport, immunohistochemistry, vitamin D, gene expression, embryonic development, in situ hybridization.

Introduction

Mammalian fetal nutrition during development is wholly dependent on the transport of nutrients by the placenta (Boyd, 1987; Hill and Longo, 1980; Munro et al. 1983; Shennan and Boyd, 1987; Truman and Ford, 1984). Calcium, needed for skeletal formation, neuromuscular functions, and other physiological activities, is transported actively across the placenta from the maternal to the fetal circulation (Brunette, 1988; Pitkin, 1985; van Kreekl and van Dijk, 1983). This process is carried out by the placental trophoblastic cells (Dearden and Ockelford, 1983; Leke and Whyte, 1985), which line the chorionic villi and represent the epithelial layer separating the maternal and fetal circulations. In the human placental chorionic villi, a layer of syncytiotrophoblasts lies over the cytotrophoblasts, and surrounds the internal mesoderm and fetal capillaries (Ramsey, 1975). Calcium transport by placental trophoblasts is therefore analogous to epithelial transport in general, i.e. calcium is moved in a transcellular manner. Furthermore, the calcium level is higher in the fetal circulation than in the maternal circulation. It is well established that calcium is actively transported against a concentration gradient by placental trophoblasts.

Our laboratory has been studying the cellular and molecular mechanism and regulation of placental calcium transport (Tuan, 1982, 1985; Tuan and Bigioni, 1990; Tuan and Cavanaugh, 1986; Tuan and Kirwin, 1988; Tuan and Kushner, 1987; Tuan et al. 1988). These studies, which are summarized below, have shown that mammalian placental trophoblasts express two marker molecules that are functional components of the calcium transport mechanism: a specific, high-Mr calcium-binding protein (CaBP) and an integral membrane Ca2+-activated ATPase. Both the CaBP and the Ca2+-ATPase are expressed as a function of embryonic development in a manner that parallels the onset of placental calcium transport. The trophoblastic localization of the CaBP and Ca2+-ATPase was revealed by immunohistochemistry and enzyme histochemistry, respectively. The functional involvement of the CaBP and Ca2+-ATPase in transmembrane calcium
transport was demonstrated using cell-free placental membrane vesicles, whose active, ATP-dependent calcium uptake was inhibited by antibodies directed against the CaBP and inhibitors of the \(Ca^{2+}\)-ATPase. Recently, a cDNA to the placental CaBP has been cloned, and has been used to probe its gene expression with respect to developmental and physiological aspects of calcium transport, and regulation of calcium handling by the JEG-3 cells.

**Materials and methods**

**Cell culture**

JEG-3 cells were obtained from the American Tissue Type Culture and were grown in RPMI 1640 (Cell-Gro) medium, 20 mM Hepes, pH 7.4, supplemented with fetal calf serum (10%) and penicillin-streptomycin, and maintained at 37°C in 5% CO\(_2\). Optimal cellular attachment was obtained with Nunc or Costar tissue culture plastic ware. In some experiments, the cultures were supplemented with 1,25-dihydroxy vitamin D\(_3\) (Biomol Research Lab., Inc.) at various concentrations for 24 h.

**Placental CaBP**

**Detection and quantitation.** Immunodetection of CaBP in JEG-3 cells was carried out using rabbit antibodies produced against the human placental CaBP (HCaBP) (Tuan, 1982, 1985). Cells were washed in phosphate-buffered saline (PBS), and homogenized in a 20 mM Tris buffer (pH 7.4), centrifuged (31,000 g, 30 min), and the soluble extract was fractionated by SDS-polyacrylamide gel electrophoresis (12% gel) (Laemmli and Favre, 1974). The gel was then electroblotted onto nitrocellulose and immunoreacted with anti-HCaBP followed by secondary antibodies conjugated with alkaline phosphatase, and then developed using a chromogenic substrate system consisting of bromochloroindolyl phosphosphate (BCIP) and nitroblue tetrazolium (NBT) (Oro and Tuan, 1990). The immunoreactive HCaBP band was scanned densitometrically using a Hoeffer scanner. The relative level of CaBP in different JEG-3 samples was determined titerimetrically on the basis of signals generated from multiple serial dilutions of each sample.

**Placental Ca\(^{2+}\)-activated ATPase**

**Detection and enzyme assay.** JEG-3 cells were extracted in a Tris buffer (pH 7.4) containing 1% Triton X-100 (Tuan and Bigioni, 1990; Tuan and Knowles, 1984; Tuan and Kushner, 1987). Total Ca\(^{2+}\)-ATPase enzyme activity, of both plasma membrane and intracellular organel, in the Trion X-100-solubilized JEG-3 extract was determined using the molybdate-Malachite Green assay as described previously (Tuan and Knowles, 1984; Tuan and Kushner, 1987), and expressed as nmol phosphate released min\(^{-1}\) mg protein\(^{-1}\).
This was measured by the Fura-2 method as reported previously. Cells were rinsed with Ca2+- and Mg2+-free Hank's Balanced Salt Solution (CMF-HBSS) to remove excess serum and calcium from the cell extract. Protein determination with [35S] methionine was performed on a non-denaturing Triton X-100 polyacrylamide gel, which was then incubated with ATP in the presence of PbCl2, followed by NaOH to precipitate the protein. The product was visualized as a dark brown band on the gel.

Protein determination

The BCA reagent of Smith et al. (1985) was used to determine protein concentrations according to the protocol provided by the manufacturer (Pierce Chemical).

Cytosolic [Ca2+] determination

This was measured by the Fura-2 method as reported previously (Akins et al.; 1988; Akins and Tuan, 1989; Gryniewicz et al., 1985). Cells were rinsed with Ca2+-, Mg2+-free Hank's Balanced Salt Solution (CMF-HBSS) to remove excess serum and calcium from the culture medium. Calcium concentration of the media was measured at 4°C for various periods of time. At the end of the incubation, the cells were rinsed in cold PBS (3 times), solubilized with 2% SDS, and the radioactivity incorporated was determined by liquid scintillation counting in Ecolume (ICN). In other experiments, calcium concentration and incubation temperature were varied. The effect of various agents was tested by pre-incubating the cells for 30 min and then measuring uptake in the presence of the respective agents. Uptake activities were expressed as pmol calcium min−1.

Cellular calcium uptake

After thorough rinsing of the tissue culture dish with Hank's Balanced Salt Solution (Ca2+-, Mg2+-free HBSS), cells were incubated with gentle agitation in HBSS containing 0.01 mM CaCl2 and trace amount of 54Ca at 37°C for various periods of time. The first procedure involved electrophoretic fractionation of the solubilized cell extract on a non-denaturing Triton X-100 polyacrylamide gel, which was then incubated with ATP in the presence of PbCl2, followed by NaOH to precipitate the protein. The reacted cells were examined with bright-field and phase-contrast optics. Photography was done using Kodak Pan-X and Ektachrome films.

Expression of placental CaBP

The presence of CaBP in JEG-3 cells was demonstrated using antibodies specific for the human placental CaBP (Tuan, 1982, 1985). Thus, both immunoblotting (Fig. 2) and immunohistochemistry (Fig. 3) revealed the presence of the CaBP. As shown in Fig. 2, a single high-Mr (~75 x 10^3) immunoreactive protein band was observed on the immunoblot, and corresponded to that found in the human term placenta. Immunohistochemistry (Fig. 3) revealed that the CaBP was associated with the cytoplasmic areas of the JEG-3 cell (Fig. 3A and B). Immunospecificity was demonstrated by the lack of staining in controls where antibodies to CaBP were omitted (Fig. 3C and D). Interestingly, not all cells found within an island stained positively for CaBP; distinct cells showing a total absence of immunostaining were observed. This finding strongly suggested that the cell population was heterogeneous with respect to CaBP expression, and further supported the notion that the cell islands were not complete syncytia.

Cultured JEG-3 cells grew as large, connecting clusters, with a well-defined border surrounding the cellular mass (Fig. 1). During the early phase of culture, the generally circular clusters (Fig. 1A and B) showed occasional fibroblast-like cellular extensions when expansion or spreading was apparent. The cells appeared mostly syncytial in the cluster, with a prominent nucleolar apparatus as well as various cytoplasmatic particles (Fig. 1C); in addition, clear vacuole-like structures were often observed along the cellular cytoplasmic cortex. Because of their syncytial nature, JEG-3 cells were routinely passaged either by simply dividing scraped cells into new cultures or lifting them off the dish with a non-enzymatic cell dissociation solution (Specialty Media Inc.). Generally, cell scraping produced a culture of initial clusters containing about 30 cells each, whereas more uniformly sized aggregates were usually obtained using the cell dissociation solution. With vigorous scraping, single cells could sometimes be released. Even during long-term culture, JEG-3 cells tended to remain as largely syncytial aggregates; however, they did not exhibit any contact inhibition, since at very high densities during long-term culture, the aggregates eventually grew on top of one another, giving rise to three-dimensional masses that were clearly visible to the naked eye. Since the placental CaBP was found to be specific for placental membranes, it was of great interest to ascertain whether it was also expressed by the JEG-3 cells, particularly if they were to be considered as potential candidate cells for the study of trophoblast calcium transport.

The expression of CaBP was analyzed at both the protein and the mRNA levels. CaBP. The presence of CaBP in JEG-3 cells was demonstrated using antibodies specific for the human placental CaBP (Tuan, 1982, 1985). Thus, both immunoblotting (Fig. 2) and immunohistochemistry (Fig. 3) revealed the presence of the CaBP. As shown in Fig. 2, a single high-Mr (~75 x 10^3) immunoreactive protein band was observed on the immunoblot, and corresponded to that found in the human term placenta. Immunohistochemistry (Fig. 3) revealed that the CaBP was associated with the cytoplasmic areas of the JEG-3 cell (Fig. 3A and B). Immunospecificity was demonstrated by the lack of staining in controls where antibodies to CaBP were omitted (Fig. 3C and D). Interestingly, not all cells found within an island stained positively for CaBP; distinct cells showing a total absence of immunostaining were observed. This finding strongly suggested that the cell population was heterogeneous with respect to CaBP expression, and further supported the notion that the cell islands were not complete syncytia.

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Fig. 1. Morphology of JEG-3 cells in culture. (A) Low magnification of hematoxylin-stained cells (bright-field optics). JEG-3 cells appeared as large multinucleated, syncytial clumps (nuclei indicated by arrowheads). (B) Low magnification with phase-contrast optics. (C) Higher magnification (phase-contrast) showing prominent nuclei (n). Bar, 100 μm (A,B) or 10 μm (C).

Fig. 2. Detection of CaBP in JEG-3 cell extract by immunoblotting. (A) Coomassie Blue-stained gel showing Mr standards (x10^-3) and protein bands in two separate extracts of JEG-3 cells. Protein load was 15 μg in each sample lane. (B) Immuno-(alkaline phosphatase) staining of nitrocellulose blot of gel shown in A. Antibodies to CaBP detected a single protein band of ~75x10^3 (arrowheads), which was similar to human term placental CaBP (Tuan, 1982).

revealed distinct cells with positive signals for the CaBP. Interestingly, as described above for immunohistochemistry of the CaBP (Fig. 3), the cDNA–mRNA hybridization signals were also heterogeneously distributed within the cell islands, since positive cells were found mingled with negative cells.

Expression of placental Ca^{2+}-ATPase
The analysis of the placental Ca^{2+}-ATPase was based on enzyme activity in solubilized JEG-3 cell extract as well as in whole cells in situ.

Cell extract. A Triton X-100-solubilized extract of the JEG-3 cells exhibited significant Ca^{2+}-activated ATPase activity at a specific activity level of 13 nmol P_i min^{-1} mg^{-1}, which was slightly lower than that reported for the human term placenta (Tuan and Kushner, 1987). The membranous nature of the enzyme activity was indicated as the activity was undetectable in non-detergent extracted cells. The enzyme activity of the JEG-3 cells was sensitive to a number of pharmacological agents (Fig. 6A), similar to that of the human placenta (Tuan and Kushner, 1987). Further evidence supporting the identity between the two enzymes was observed upon electrophoretic fractionation of the activities (Fig. 6B). Thus, histochemical detection of enzyme activity on a non-denaturing electrophoretic gel showed that JEG-3 cells and placental microsomes contained an activity band with identical electrophoretic mobility, which corresponded to that previously identified as associated with trophoblasts (Tuan and Kushner, 1987).

Enzyme cytohistochemistry. Ca^{2+}-ATPase activity was also detected histochemically in paraformaldehyde-fixed JEG-3 cells (Fig. 7A,B). The staining was most intense in the cellular periphery and in general appeared to be...
Fig. 3. Immunohistochemical localization of CaBP in JEG-3 cells. Paraformaldehyde-fixed cells were immunostained with anti-CaBP antibodies as described in Materials and methods. (A,B) Immunostaining for CaBP (arrowheads in A, arrows in B) was seen clearly associated with a group of syncytial cells on the left of the micrograph, whereas other syncytial cells located at the right and bottom (open arrows) were negative. A. Phase-contrast optics; B, epifluorescence optics. (C,D) Control with the omission of antibodies to CaBP, showing the specificity of the immunostaining in A and B. C. Phase-contrast optics; D, epifluorescence optics. (E,F) CaBP immunostaining of JEG-3 cells treated in culture with (F) or without (E) 0.5 nM 1,25-dihydroxy vitamin D₃ for 24 h. Epifluorescence optics for both E and F. Note the significantly elevated staining signal in the treated culture. Bar, 10 μm. Magnification identical for A–D and E–F, respectively.
Calcium uptake activity of JEG-3 cells

JEG-3 cells exhibited active uptake of extracellular calcium under the experimental conditions described in Materials and methods. The characteristics of JEG-3 calcium uptake are shown in Fig. 8A and B. Thus, calcium uptake was temperature-dependent and exhibited near-linear kinetics at 37°C for at least 7–8 min (Fig. 8A). The uptake activity also increased proportionally with medium calcium concentration (Fig. 8B). Calcium uptake was dependent on the viability and membrane integrity of the JEG-3 cells, as paraformaldehyde fixation and digitonin treatment both significantly reduced calcium uptake activity (data not shown). In addition, JEG-3 calcium uptake was sensitive to a number of pharmacological agents. As shown in Fig. 8C, agents that were previously found to inhibit the Ca^{2+}-ATPase activity (Fig. 6A), in particular quercetin and phenothiazin also effectively reduced calcium uptake by the JEG-3 cells. However, erythrosin B, which strongly inhibited Ca^{2+}-ATPase activity (Fig. 6A), was ineffective on cellular calcium uptake (Fig. 8C), possibly due to membrane impermeability of the drug. Overall, the similarity between the pharmacological sensitivity of the calcium uptake and Ca^{2+}-ATPase enzyme activities of the JEG-3 cells strongly suggested that these two activities might be related.

To gain insight into the process of calcium handling by the JEG-3 cells during active calcium uptake, cytosolic [Ca^{2+}] was analyzed by Fura-2 microfluorometry. As

![Fig. 4. Detection of CaBP mRNA in JEG-3 cells by Northern RNA blotting. Autoradiograms obtained after hybridization with CaBP cDNA probe (A), and hybridization of the same blot with β-actin cDNA probe (B). Lane 1, term placenta total RNA; lane 2, JEG-3 total RNA. Each lane contained approximately 10 µg of RNA. The blot was calibrated with size markers (kb=10^3 bases) as indicated; the electrophoretic mobilities of rRNAs are also indicated. A 2.96 kb CaBP mRNA band (arrow) was detected in both term placenta and JEG-3, whereas a 2.3 kb band was seen with the β-actin probe.](image)

![Fig. 6. Characterization of Ca^{2+}-ATPase activity of JEG-3 cells. (A) Effect of various pharmacological agents; and (B) electrophoretic mobility on denaturing gel. In A, solubilized JEG-3 extract was first incubated with the respective pharmacological agents at the indicated concentrations for 10–15 min and then assayed in the presence of the agents at the same concentration. All activities are expressed as a percentage of the control in the absence of any agents. Values represent the mean of 2–3 experiments. All agents significantly inhibited enzyme activity (P<0.05). In B, solubilized extracts of JEG-3 membranes and term placental microsomes, prepared as described by Tuan and Kushner (1987), were subjected to Triton X-100 nondenaturing polyacrylamide (8 %) gel electrophoresis and stained histochemically for Ca^{2+}-ATPase activity as described in Materials and methods. Lane J, JEG-3; lane P, placenta. Left panel, histochemical reaction in the presence of 1 mM CaCl2; middle panel, in the absence of CaCl2; and right panel, Coomassie Blue protein staining profile. Protein load per lane: JEG-3, 0.55 µg for histochemistry, 2.8 µg for Coomassie Blue staining; placental microsomes, 0.18 and 0.39 µg, respectively. Note the identical Ca^{2+}-ATPase activity band seen in both JEG-3 and placenta (arrowheads).](image)
Fig. 5. Localization of CaBP mRNA in JEG-3 cells by in situ hybridization. Biotinylated pMCP was used as the probe, and was localized after hybridization by means of alkaline phosphatase histochemistry as described in Materials and methods. (A) Phase-contrast optics; (B) Nomarski differential interference contrast optics. Positive hybridization was seen as purple stain associated with the cell body. Note the localization of CaBP mRNA transcripts within several syncytial structures (arrows), and the absence of signal in other cells (open arrow). Bar, 10 μm.
Fig. 7. Cytohistochemical localization of Ca\(^{2+}\)-ATPase in JEG-3 cells. This was carried out as described in Materials and methods. A,B. The brownish-colored reaction product is shown in color (phase-contrast optics; A, before reaction; B, after reaction); see localization to the periphery of cells in high density areas (arrows). Negative cells were also seen (open arrows). (C–K) Black-and-white micrographs of enzyme histochemistry under various incubation conditions. (C–E) +Ca\(^{2+}\), +ATP; (F–H) -Ca\(^{2+}\), +ATP; and (I–K) +Ca\(^{2+}\), +AMP. Paraformaldehyde-fixed cells were first observed with phase-contrast (C,F,I) and bright-field (D,G,J) optics after Ca\(^{2+}\)-ATPase reaction but prior to the addition of Na\(_2\)S. The reaction product was then visualized as PbS precipitates and observed by bright-field optics (E,H,K). Positive reaction was only seen in the presence of both calcium and ATP (E); both positive cells (arrowheads) and negative cells (open arrow) were evident. Bar, 10 μm.
shown in Fig. 9, washed JEG-3 cells exposed to external calcium (5 mM) showed an immediate, rapid, and transient rise in cytosolic [Ca^{2+}], preceding by a considerable time interval the actual cellular uptake of 46Ca (Fig. 8A). In fact, cytosolic [Ca^{2+}] rapidly decayed to a plateau only slightly higher than the starting value. In addition, 0.5 nM 1,25-dihydroxy vitamin D$_3$ treatment, which greatly enhanced calcium uptake (see below), did not significantly alter the cytosolic [Ca^{2+}] profile (Fig. 9B). From this kinetic analysis, it appeared unlikely that the calcium uptake activity of JEG-3 cells could be accounted for simply by a rise in cytosolic free [Ca^{2+}]; an alternative calcium sequestration mechanism was thus necessary.

Fig. 9. Kinetic changes in cytosolic [Ca^{2+}] during calcium uptake by JEG-3 cells. Cytosolic [Ca^{2+}] was estimated by Fura-2 microfluorimetry and expressed here as the ratio of the excitation intensity at 351 nm to that at 380 nm, as described in Materials and methods. JEG-3 cells were pre-incubated with 1 mM EGTA and then exposed to CaCl$_2$. [Ca^{2+}] was calculated using the formula of Grynkiewicz et al. (1985). (A) Control JEG-3 cells, perfused with 5 mM CaCl$_2$; (B) JEG-3 cells treated with 0.5 nM 1,25-dihydroxy vitamin D$_3$ for 24 h (see legend to Fig. 10), perfused with 2 mM CaCl$_2$. The profiles shown here are representative of those observed in typical experiments, where baseline [Ca^{2+}]=15-20 nM, peak [Ca^{2+}]=~600 nM, and plateau [Ca^{2+}]=190-200 nM. Arrowheads indicate beginning of perfusion.

Vitamin D regulation of calcium uptake and CaBP expression by JEG-3 cells
To investigate the effect of 1,25-dihydroxy vitamin D$_3$, JEG-3 cells were treated with the hormone at various doses for 24 h and then analyzed for their calcium uptake activity and level of CaBP expression. As shown in Fig. 10A, 1,25-dihydroxy vitamin D$_3$ treatment significantly stimulated JEG-3 calcium uptake in a dose-dependent manner, the stimulation being maximal, 200%, at 0.5 nM. Interestingly, the enhancement of cellular calcium uptake was accompanied by a concomitant increase in the steady-state level of the CaBP, as determined by quantitative immunoblotting. The results in Fig. 10B, based on densitometry of the immunoreactive CaBP band on a Western blot, clearly indicated that 1,25-dihydroxy vitamin D$_3$ treatment stimulated CaBP by approximately twofold, compared to control.
Significant stimulation was seen at 0.5 nM. Results are based on the slope of the titration curve, was seen in JEG-3 cells. Immunoquantitation of CaBP was carried out by Western blot analysis as described in Materials and Methods. Calcium uptake as described in Materials and Methods. The biochemical activities of the JEG-3 cells mimic those of the term placental trophoblasts, with respect to the CaBP and Ca²⁺-ATPase, both of which appear to be identical to their placental counterparts. Thus, the JEG-3 CaBP is immunoreactive with anti-human CaBP antibodies and has identical \( M_r \) and subcellular distribution with human placental CaBP; furthermore, the electrophoretic mobility and pharmacological sensitivity of the two Ca²⁺-ATPase activities are also similar. (Note: total cellular Ca²⁺-ATPase activities are analyzed, and thus both plasma membrane and intracellular activities are compared.) In addition, previous studies have also firmly established that the endocrinological (Kohler and Bridson, 1971; Patillo and Gey, 1968) and biochemical (Bahn et al. 1981; Hamilton et al. 1979) properties of the JEG-3 cells are similar to those of the placental syncytiotrophoblasts.

In this study we have characterized calcium uptake by JEG-3 cells cultured on tissue culture plastic. The substrate and temperature dependence, linear kinetics and membrane integrity requirement of the uptake process are all consistent with an energy-requiring, cellular uptake process. Interestingly, uptake activity is stimulated by 1,25-dihydroxy vitamin \( D_3 \) treatment of the JEG-3 cells, which also significantly increases the level of the cytosolic CaBP, but not the Ca²⁺-ATPase. It should be noted that calcium uptake as measured here is a net result of influx and subsequent efflux of extracellular \(^{45}\)Ca. Consequently, it is reasonable to speculate that the effect of 1,25-dihydroxy vitamin \( D_3 \) may be, first, to enhance the expression of the cytosolic CaBP, which then acts to increase retention of the influxed \(^{45}\)Ca. Thus, the high-\( M_r \), CaBP of the placental trophoblasts may function in a manner similar to that of the low-\( M_r \) calcium-binding protein, calbindin-9K. Calbindin, first discovered as the vitamin D-dependent calcium-binding protein of the intestinal mucosa (Kallfelz et al. 1967; Marche et al. 1977), is present in the placenta (Bruns et al. 1978; Marche et al. 1978) and appears to increase in level as a function of gestation (Delorme et al. 1979). Various studies have suggested that calbindin may act as a cytosolic calcium

Discussion

We have characterized the human choriocarcinoma cell line, JEG-3, as a candidate in vitro system for the study of placental calcium transport. The results reported here strongly indicate that the JEG-3 cells mimic placental trophoblasts with respect to many of the characteristics and biochemical activities associated with calcium transport. These include the expression of two previously identified marker molecules, a high-\( M_r \), CaBP and a Ca²⁺- activated ATPase, temperature- and substrate-dependent and kinetically linear calcium uptake, and responsiveness to 1,25-dihydroxy vitamin \( D_3 \). Taken together with the syncytial morphology of the JEG-3 cells, which resembles that of the syncytiotrophoblasts, these findings support the validity of these cells as an in vitro model for studying placental calcium transport.

The important function of the placenta as the sole tissue responsible for nutrient translocation from the maternal to fetal circulation depends on the developmental differentiation of the trophoblasts into a specialized transporting epithelium (Dearden and Ockleford, 1983; Loke and Whyte, 1983). Thus, the syncytiotrophoblasts constitute a thin, tight epithelial interface between the maternal and fetal circulations. Detailed understanding of the mechanism of transport therefore requires studying the trophoblasts as an isolated cellular epithelium. Ideally, this should be a defined cell line that may be propagated reproducibly and also continuously express properties characteristic of the trophoblasts. The findings reported here are thus significant, since they establish the JEG-3 cells, a stable cell line, as an in vitro experimental model of placental calcium transport.
sink to facilitate transepithelial calcium transport (Carafoli, 1987; Wasserman and Fullmer, 1983), particularly in the intestinal mucosa, although its exact functional role remains to be established. It is thus noteworthy that in the JEG-3 cells, total cytosolic [Ca] did not rise concomitantly with calcium uptake, implicating a possible role for the CaBP as an intracellular calcium sequestor. A number of recent studies have also implicated calbindin as functionally involved in placental transport; in addition, another calcium-binding protein, oncomodulin (Brewer and MacManus, 1985), first identified in transformed cells, has also been identified in the placenta. How all these proteins may participate in calcium transport, or possibly other metabolic functions of the placenta, remains to be elucidated. Nevertheless, the regulatory role of 1,25-dihydroxy vitamin D in placental calcium transport is strong suggested, since both the high-M, CaBP studied here and calbindin-9K are vitamin D-dependent. It should be noted that, unlike calbindin-9K (Mathieu et al., 1989), the vitamin D-stimulated increase in the level of CaBP appears to be accompanied by a concomitant increase in mRNA. The exact mechanism of vitamin D stimulation of CaBP expression therefore remains to be established.

Finally, the study of transepithelial transport in vitro ideally requires the use of a tight, polarized cellular epithelial sheet that will mimic the vectorial translocation of solutes (Almers and Stirling, 1984; Rodriguez-Boulan and Nelson, 1988; Sabatini et al. 1983; Simons and Fuller, 1985), such as the widely studied MDCK cell line. As described earlier (Fig. 1), the JEG-3 cells will develop in long-term culture into a cellular sheet consisting of overlapping cell clusters. Although these cell islands are heterogeneous with respect to the expression of the CaBP and Ca-ATPase, the fact that they form a cell sheet is a distinct advantage in the study of transepithelial transport. Experiments are underway to examine the tightness and the polarity of the JEG-3 cell sheet. Initial observations on JEG-3 cells cultured on permeable membrane substrata suggest that a tight epithelium is indeed formed in vitro.

In summary, the studies presented here have provided a firm basis for the application of the human choriocarcinoma cell line, JEG-3, as an in vitro cellular model for the study of calcium transport by placental trophoblasts. In particular, the phenotypic stability and the hormonally responsiveness of these cells should greatly facilitate the understanding of the mechanism and regulation of trophoblast calcium transport.

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