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Evidence for a Zinc Uptake Transporter in Human Prostate Cancer Cells Which Is Regulated by Prolactin and Testosterone*

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The glandular epithelial cells of the human prostate gland have the unique capability and function of accumulating the highest zinc levels of any soft tissue in the body. Zinc accumulation in the prostate is regulated by prolactin and testosterone; however, little information is available concerning the mechanisms associated with zinc accumulation and its regulation in prostate epithelial cells. In the present studies the uptake and accumulation of zinc were determined in the human malignant prostate cell lines LNCaP and PC-3. The results demonstrate that LNCaP cells and PC-3 cells possess the unique capability of accumulating high levels of zinc. Zinc accumulation in both cell types is stimulated by physiological concentrations of prolactin and testosterone. The studies reveal that these cells contain a rapid zinc uptake process indicative of a plasma membrane zinc transporter. Initial kinetic studies demonstrate that the rapid uptake of zinc is effective under physiological conditions that reflect the total and mobile zinc levels in circulation. Correspondingly, genetic studies demonstrate the expression of a ZIP family zinc uptake transporter in both LNCaP and PC-3 cells. The rapid zinc uptake transport process is stimulated by treatment of cells with physiological levels of prolactin and testosterone, which possibly is the result of the regulation of the *ZIP*-type zinc transporter gene. These zinc-accumulating characteristics are specific for prostate cells. The studies support the concept that these prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transporter gene associated with their unique ability to accumulate high zinc levels.

Zinc is an essential component of all cells. It is required for a variety of cellular activities such as metalloenzyme activity, nucleoprotein and nucleic acid structure, and transcription factor interactions. Typically, intracellular zinc is found predominantly (> 95%) bound to high molecular weight ligands such as metalloenzymes, metalloproteins, nucleoproteins, and nucleic acids. Very little zinc is available as free or loosely bound zinc, which we will refer to as " mobile reactive zinc" (for review, see Refs. 1–3).

The prostate gland of humans and other animals is unique in that it accumulates much higher zinc levels than any other soft tissues in the body. For detailed and extensive reviews of zinc-citrate relationships in prostate, see Refs. 4–6. The special functions associated with the high zinc level of the prostate have not been resolved. The ability of the prostate to accumulate high zinc levels is a function of the glandular secretory epithelial cells. Our recent studies (7) with rat prostate lobes have demonstrated that the epithelial cells contain high levels of intracellular zinc, and, most importantly, contain high levels of mitochondrial zinc. The accumulation of zinc results in the inhibition of mitochondrial aconitase activity which minimizes the ability of these cells to oxidize citrate (8). This is an important relationship associated with the unique functional and metabolic capability of the prostate to accumulate high citrate levels. This results in a significant portion (about 30%) of the total zinc of citrate-producing prostate cells existing in a chelated form with citrate (9–11), which is in contrast to other cells in which greater than 95% of the intracellular zinc is bound to macromolecules in an immobile form.

Animal studies have revealed that the accumulation of zinc in the prostate is regulated by testosterone and prolactin (6, 7, 12). In rats, both hormones increase the cellular and mitochondrial levels of zinc in lateral prostate cells; both hormones decrease the cellular and mitochondrial levels of zinc in ventral prostate cells; and neither hormone has any effect on the zinc levels of dorsal prostate cells or non-prostate cells (7). Dorsal prostate and non-prostate cells are not citrate-producing cells, whereas lateral and ventral prostate cells are citrate-producing cells. This raised the important question as to which types of epithelial cell exist in the human prostate in relation to its function of producing, accumulating, and secreting extremely high levels of zinc. Moreover, it is well established that naturally occurring malignant prostate cells lose the ability to accumulate zinc. Despite these important functional and pathological relationships, virtually no information exists concerning the mechanism(s) involved in the accumulation of zinc and its regulation in human prostate epithelial cells. The present report reveals that 1) LNCaP and PC-3 cells (human malignant prostate cell lines) exhibit the capability of accumulating high zinc levels; 2) a rapid zinc uptake transport mechanism is associated with the accumulation of high zinc levels; 3) prolactin and testosterone are positive regulators of the transport mechanism; and 4) LNCaP and PC-3 cells express a hormonally regulated ZIP-type plasma membrane zinc uptake transporter.

EXPERIMENTAL PROCEDURES

For these studies we elected to employ the human malignant cell lines LNCaP and PC-3. As presented under "Results," both cell lines exhibit the capability of accumulating high zinc levels under the *in vitro* conditions employed; therefore, they are excellent models for studying the mechanisms and regulation of zinc accumulation. Moreover, LNCaP cells exhibit the characteristics of citrate-producing cells with a limiting *m*-aconitase, whereas PC-3 cells are citrate-oxidizing cells in which *m*-aconitase is not limiting (13). In addition, LNCaP cells have a very

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low tumorigenicity compared with the highly tumorigenic PC-3 cells. Thus, comparative studies in these cell types will offer the future advantage of establishing the zinc relationships to citrate metabolism and malignancy.

The conditions for culture and harvesting of the cells were the same as described previously (13). The culture medium was RPMI 1640 and 10% fetal bovine serum (FBS).[1] Generally, at 18 h before cell harvesting, the medium was changed to RPMI 1640 without the addition of FBS to minimize any effect of unidentified components of FBS which might influence the accumulation of zinc. The harvested cells were washed and suspended in Hanks' balanced salt solution (HBSS) and used in the experiments. Transfection of PC-3 cells with androgen receptor was achieved as described previously (14).

For experiments involving total zinc accumulation, the incubation medium was HBSS. Depending upon the experiment, zinc and hormones were added to the medium. After an appropriate incubation period at 37 °C, the cells were quickly centrifuged and washed with HBSS. The washed cells were then digested and prepared for atomic absorption assay of zinc as described previously (7). The cellular zinc levels were calculated as ng/mg cell protein. Cell protein was assayed by the method of Bradford (15).

Zinc transport was determined by ^{65}Zn rapid uptake by the cells. In these experiments the harvested cells ($2–5 \times 10^6$) were added to microcentrifuge tubes. Generally, 200 μl of HBSS containing $^{65}ZnCl_2$ was added to the microcentrifuge tubes. After the appropriate incubation period, 1.5 ml of cold stop solution (250 mM sucrose containing 1 mM EDTA and 50 mM Hepes buffer, pH = 7.2) was rapidly added to the microcentrifuge tubes followed by rapid centrifugation. The packed cells were rapidly resuspended in 1.5 ml of cold stop solution and centrifuged. The packed cells were collected in liquid scintillation mixture and ^{65}Zn counted in a liquid scintillation counter.

The following procedures were employed to determine the expression of a plasma membrane zinc uptake transporter in the prostate cells. A ZIP family of metal transporters which includes zinc transporters has been identified in plant and yeast cells (16–19). A putative mammalian zinc transporter (H2O615) has been identified in the Expressed Sequence Tags data base which shares remarkable homology with the *Arabidopsis* ZIP1. ZIP1 is a plasma membrane zinc uptake transporter, and therefore was considered by us to be a prime candidate for expression in prostate cells. The partial cDNA clone (H2O615) was used to screen the Human Universal cDNA Library Array (HUCL) from the Stratagene Corporation. We identified a cDNA clone in the HUCL which hybridized, under high stringency conditions, with the EST partial clone. We designated this HUCL clone "*hZIP1*" (human *ZIP1*) which was used to determine the expression of the putative zinc uptake transporter in LNCaP and PC-3 cells. The procedures for RNA extraction and Northern blot analysis for the detection of *hZIP1* mRNA have been described (20).

The zinc accumulation and kinetic experiments were repeated two or more times to ensure the reproducibility of the results, and all assays were run in duplicate or triplicate depending upon the volume of sample available for assay. Without exception, the standard errors within each experiment were less than 5% of the mean values presented under "Results." Data presented as statistically significant represent a $p < 0.05$, although most of the statistically significant differences exhibited $p < 0.01$ values. The reproducibility and consistency of the results are demonstrated by the fact that no repeated experiment deviated from the typical effects presented under "Results."

RESULTS

In the first series of studies we had to determine if LNCaP cells and/or PC-3 cells exhibited the capability of accumulating high zinc levels, which would be representative of the unique zinc-accumulating characteristic of the human prostate. The endogenous level of cellular zinc in LNCaP and PC-3 cells and the accumulation of zinc in cells exposed to zinc added to the medium were established (Table I). The "endogenous " level is defined as the concentration in the cells grown and maintained in unsupplemented medium with respect to zinc. We assayed the media and found that RPMI/FBS contains about 0.05 μg zinc/ml; when FBS is omitted, the RPMI medium as well as HBSS contains no detectable zinc. The mean endogenous level of zinc in LNCaP cells (248 ng/mg protein) was 51% higher than the level (164 ng/mg protein) in PC-3 cells. Throughout these studies and without exception the endogenous cellular zinc concentration of LNCaP cells was consistently higher than the corresponding zinc level of PC-3 cells. Consequently it is clear that LNCaP cells contain a significantly higher endogenous zinc level than PC-3 cells. When the harvested cells were incubated for 4 h in HBSS medium supplemented with 1 μg/ml zinc (the approximate concentration in plasma) in the form of zinc sulfate, the cellular level of zinc in LNCaP increased by 98% to 493 ng/mg protein; and PC-3 zinc levels increased 118% to 358 ng/mg protein. Thus, both LNCaP and PC-3 cells took up and accumulated zinc from the medium; however, LNCaP cells maintained significantly higher (37%) zinc levels than PC-3 cells in the presence of physiological exogenous zinc levels. In experiment 3 (Table I) we had also included (not shown) zinc measurements in squamous carcinoma cells to represent a non-prostate cell type. The zinc level of the squamous carcinoma cells was 174 ng/mg protein compared with 428 and 306 for LNCaP and PC-3 cells, respectively. Thus, the zinc level of the non-prostate cells was significantly less than the prostate cells. This correlates with the rat studies in which non-prostate cells (kidney and liver cells) contain significantly lower zinc levels than prostate cells (7).

TABLE I
Total cellular zinc levels of LNCaP and PC-3 cells

The cells were grown in RPMI 1640 and 10% FBS. The medium was changed to RPMI 1640 at 18 h before harvesting. The harvested cells were incubated for 4 h in HBSS (−zinc) or HBSS containing 1 μg zinc/ml (+zinc). The values are the means (±S.E.) expressed as ng of zinc/mg of cell protein.

	LNCaP		PC-3	
	−Zinc	+Zinc	−Zinc	+Zinc
Exp. 1	255 (11)	561 (23)[a]	167 (8)	486 (19)[a]
Exp. 2	239 (10)		147 (6)	
Exp. 3		428 (20)		306 (14)
Exp. 4	250 (11)	492 (22)[a]	178 (8)	283 (14)[a]
Mean ± S.E.	248 (5)	493 (34)[a]	164 (9)	358 (64)[a]

[a] Significant difference between +zinc and −zinc for LNCaP and PC-3 cells.

We then determined the effects of prolactin and testosterone treatment of the cells on zinc accumulation (Table II). In these experiments the harvested cells were incubated for 4 h in HBSS medium containing 1 μg/ml zinc and 30 nM prolactin, 10 nM testosterone, or vehicle (control). With LNCaP cells, zinc accumulation was increased significantly by both testosterone (+51%) and prolactin (+37%). With PC-3 cells, zinc accumulation was increased significantly by prolactin (+40%), but testosterone had no significant effect. The absence of an effect of testosterone on PC-3 cells might be expected because these cells do not contain androgen receptor; therefore we determined the effect of testosterone on PC-3 cells that were transfected with androgen receptor (Table III). Testosterone treatment increased the accumulation of zinc in the transfected cells by 32% but had no effect on the nontransfected PC-3 cells. Moreover, the zinc levels of untreated transfected and nontransfected cells were the same, so transfection alone had no effect on zinc accumulation. The transfection result is also important because it indicates that the testosterone effect is mediated via a gene-regulated mechanism. Consequently the effect of actinomycin on the hormonal stimulation of zinc accumulation was determined (Table II). With LNCaP cells actinomycin completely inhibited the stimulatory effect of both prolactin and testosterone. With PC-3 cells actinomycin inhibited the effect of prolactin. Because the PC-3 cells were not transfected, testosterone had no effect on zinc accumulation, and

[1] The abbreviations used are: FBS, fetal bovine serum; HBSS, Hanks' balanced saline solution; HUCL, Human Universal cDNA Library; PRL, prolactin.

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TABLE II

Effects of prolactin and testosterone on zinc accumulation in LNCaP and PC-3 cells

Cells were incubated in HBSS containing 1 μg/ml zinc plus 30 nM prolactin or 10 nM testosterone or vehicle (control), with or without 8 μM actinomycin. The values are the means (±S.E.) reported as ng of zinc/mg of protein. Cont., control; +Test., testosterone added; Act., actinomycin; + PRL, prolactin added.

	LNCaP			PC-3		
	Cont.	+PRL	+Test.	Cont.	+PRL	+Test.
−Act.	428 (21)	586 (29)[a]	650 (32)[a]	306 (14)	429 (18)[a]	334 (13)
+Act.	428 (24)	450 (22)	458 (19)	306 (12)	309 (12)	309 (11)

[a] Significant difference *versus* control.

TABLE III

Effect of androgen receptor transfection on testosterone stimulation of zinc accumulation in PC-3 cells

The conditions are the same as presented in Table II. The values are the means (±S.E.) expressed as ng of zinc/mg of protein. [% change from control.]

Nontransfected		Transfected	
Control	Testosterone	Control	Testosterone
397 (14)	425 (19) [+7%]	388 (16)	513 (22) [+32%][a]

[a] Significant difference *versus* control.

actinomycin had no effect. The results indicate that the stimulatory effects of prolactin and testosterone on zinc accumulation involve some aspect of gene expression. Neither testosterone nor prolactin treatment had any effect on the zinc levels of squamous carcinoma cells cells (results not shown); therefore the hormonal effects on the prostate cells exhibit cell specificity.

The protocol for the experiments described above involved the simultaneous incubation of harvested cells for 4 h in medium containing 1 μg/ml zinc and hormone; however it was now important to establish how early the cellular accumulation of zinc would be manifested. To eliminate the hormone induction period from the zinc uptake kinetics, PC-3 cells were pretreated with prolactin-supplemented RPMI medium overnight to maximize the prolactin effect compared with cells maintained in prolactin-free RPMI. The treated and control cells were harvested, washed, and incubated in HBSS containing 1 μg/ml zinc over a period of 60 min. The results (Fig. 1) demonstrate that a prolactin-induced increase in zinc accumulation was clearly evident by 60 min. Of special importance is the magnitude of the difference in zinc accumulation by 60 min associated with the hormone effect, a difference of 109 ng/mg protein, which is a 33% increase in total zinc. This is important because less than 5% of the total cellular zinc of mammalian cells is represented by mobile (free or loosely bound) reactive zinc, which would calculate to approximately 20 ng/mg protein. Thus, the difference of 109 ng/mg protein would actually represent an increase of 5-fold in relation to mobile zinc. Obviously, the magnitude of this increase would require that the increase in zinc accumulation must have been initiated very early, probably within minutes, in order for this accumulated difference to be so large by 60 min.

The above results indicated that the accumulation of zinc involved a rapid zinc uptake process. To determine this possibility we determined the rapid uptake of ^{65}Zn in PC-3 cells. The results (Fig. 2) demonstrate the existence of a rapid uptake mechanism. The zinc uptake rate was greatest within the first 30 s, and the rate declined thereafter. If this rapid uptake process were linked to the zinc accumulation in these cells, we reasoned that the rapid uptake of zinc should exist in both cell types and should be hormone-responsive as is the case with zinc accumulation. In these studies, the harvested cells were divided into two groups: a group that was pretreated by incubation for 3 h in medium containing 30 nM prolactin *versus* a control group containing hormone vehicle. The results (Fig. 3)

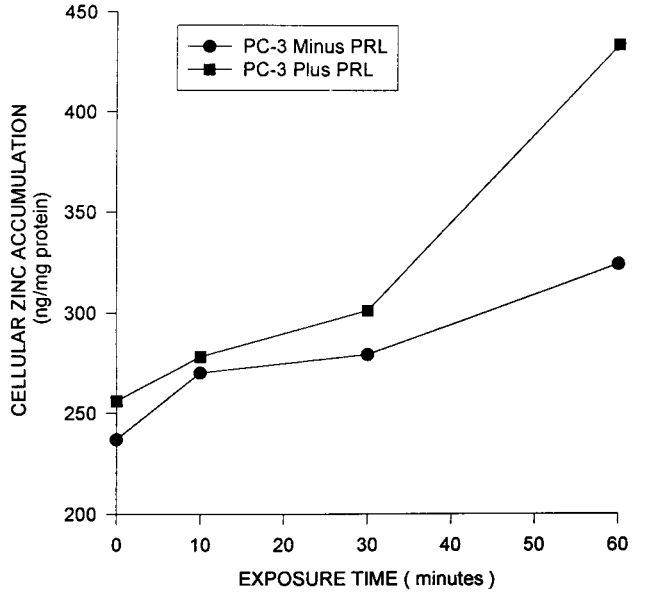

FIG. 1. **Effect of prolactin pretreatment of PC-3 cells on the accumulation of zinc.** The cells were exposed for 18 h to RPMI medium containing either 30 nM prolactin or vehicle (minus prolactin). The pretreated cells were harvested and incubated in HBSS containing 1 μg/ml zinc for periods up to 60 min.

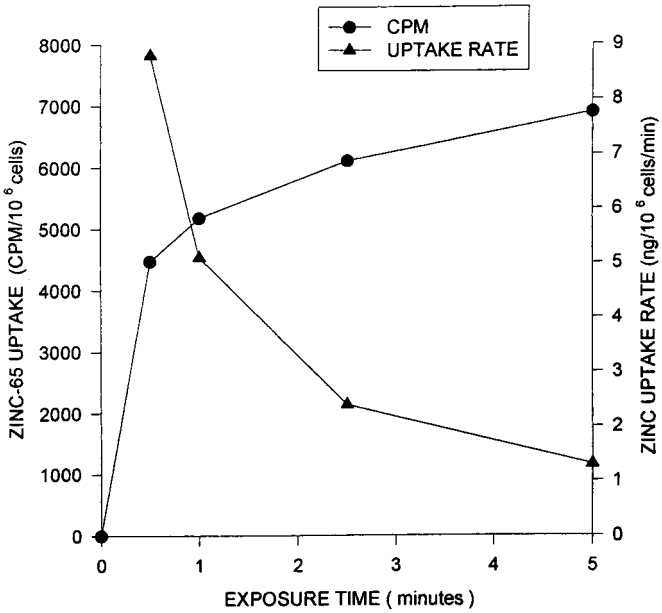

FIG. 2. **Rapid uptake of zinc by PC-3 cells.** PC-3 cells were incubated in HBSS containing 0.5 μg/ml zinc labeled with ^{65}Zn.

demonstrate that LNCaP cells also exhibited the existence of a rapid zinc transport mechanism; however, the rapid uptake rate of LNCaP cells was 3–5 times greater than PC-3 cells, which is consistent with higher zinc accumulation in LNCaP

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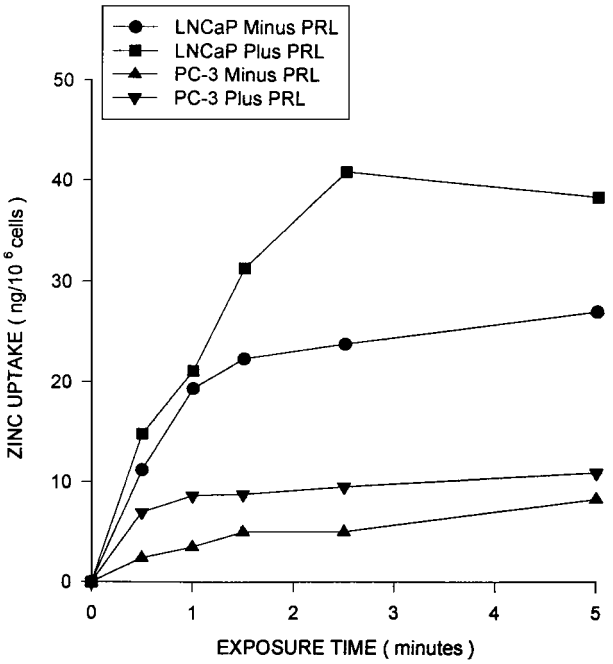

FIG. 3. **Effect of prolactin pretreatment of LNCaP and PC-3 cells on the rapid uptake of zinc.** Cells were pretreated for 3 h with HBSS containing 30 nM prolactin, 10 nM testosterone, or vehicle. The pretreated cells were harvested and incubated in HBSS medium containing 1 μg/ml zinc labeled with ^{65}Zn.

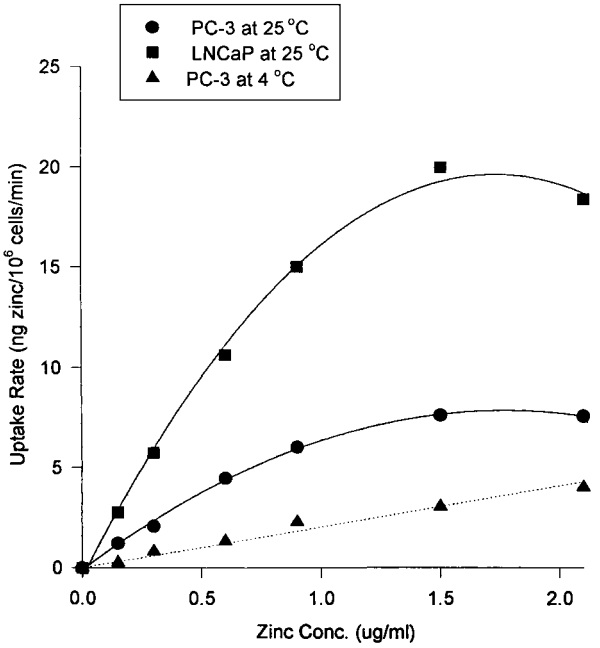

FIG. 4. **Rapid uptake of zinc by LNCaP and PC-3 cells at physiological levels of zinc.** The uptake rates at 4 °C reflect mainly the net diffusion of zinc into the cells. The difference in zinc uptake at 4 °C and at 25 °C reflects the rapid transport of zinc into the cells.

cells compared with PC-3 cells. Prolactin pretreatment markedly stimulated the rapid uptake of zinc by LNCaP and PC-3 cells. The effect is more pronounced in LNCaP cells, but in both cases the hormonal increase was about 2-fold. Moreover, the effect of the hormone appeared to involve an increase in the maximal rate of zinc uptake. This too is indicative of an effect on a zinc transporter. The prolactin-induced effect within a 3-h exposure time to prolactin is the same time frame involved in maximal prolactin stimulation of other gene-regulated metabolic activities of prostate cells (4–6).

In plasma the total zinc concentration is about 1 μg/ml. Of this total, about 34% is firmly bound (mainly to globulin), and 66% is loosely bound, which represents the "mobile" component available for cellular uptake; therefore the rapid uptake of zinc by LNCaP and PC-3 cells over the range of 0.1–2.0 μg/ml zinc was determined (Fig. 4). Rapid zinc uptake at 25 °C by LNCaP and PC-3 cells was increased as the extracellular concentration of zinc was increased up to approximately 1.0 μg of zinc/ml. The maximal uptake rate for both cells occurred at about 1.5 μg of zinc/ml. It is also apparent that the rapid uptake process was saturable, which is representative of either an active or facilitative transport process. The rapid transport rates at zinc levels representative of circulating levels of mobile zinc are about three times greater for LNCaP than PC-3 cells. This is consistent with the results described above and is consistent with the capability of LNCaP cells to accumulate higher zinc levels than PC-3 cells. Because of the low ^{65}Zn uptake rates at this low physiological zinc concentration range, we established the diffusion of zinc into the cells. For this we compared the ^{65}Zn rapid uptake of PC-3 cells at 4 and 25 °C (Fig. 4). At 4 °C, the rapid zinc uptake should be the result mainly of the diffusion of zinc into the cells with the zinc uptake by transport being minimal. This is evident in that zinc uptake at 4 °C was linear over the zinc concentration range and exhibited no saturation kinetics. It is readily apparent that the influx of zinc at 4 °C over this physiological range of was minimal in relation to the uptake of zinc at 25 °C. Even at extremely low zinc concentrations (*e.g.* 0.15–0.3 μg/ml) the rapid transport of zinc into the cells was evident. At the expected normal circulating range of diffusable zinc (0.3–0.9 μg/ml), the cellular uptake of zinc by transport was increased over the diffusion of zinc by 3-fold for PC-3 cells and by 9-fold for LNCaP cells. Collectively these studies demonstrate the existence of a rapid zinc uptake transporter in the prostate cells which is capable of functioning at zinc levels representative of plasma zinc concentrations. We are now conducting kinetic studies to establish the optimal conditions for this rapid zinc transport at which time we will be able to determine accurately the K_m and V_{max} for this transporter and the effects of hormonal stimulation on the kinetics.

In all of the hormonal studies described above, 10 nM testosterone and 30 nM prolactin were employed, which proved effective to stimulate zinc uptake in the prostate cells. The normal circulating levels of prolactin and testosterone are approximately 2 nM and 1 nM, respectively. It was important to determine if lower hormonal concentrations were effective in stimulating zinc uptake. Table IV demonstrates that prolactin as low as 0.6 nM significantly stimulated rapid zinc uptake of LNCaP cells, and a dose response occurred as the concentration of prolactin was increased. Testosterone at a concentration of 0.1 nM significantly increased the rapid zinc uptake, and a dose response existed. With PC-3 cells, 0.6 nM prolactin also stimulated rapid zinc uptake, while testosterone had no effect as would be expected in these androgen receptor-deficient cells. It is of interest to note that prolactin had a more pronounced effect on stimulation of the rapid uptake of zinc in PC-3 cells compared with LNCaP cells, although LNCaP cells accumulate higher zinc levels. This might be because of the possibility that the rapid transport mechanism is more active in the LNCaP cells in the absence of added hormone, which contributes to the higher endogenous zinc levels of these cells. Nevertheless, these studies demonstrate that both prolactin and testosterone at low physiological levels are effective regulators of zinc uptake in prostate cells. This is consistent with the rat studies in which the combination of *in vivo* and *in vitro* experiments demonstrated that prolactin and testosterone are physiological regulators of zinc accumulation in prostate (7).

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TABLE IV
Effects of varying concentrations of prolactin and testosterone on the rapid uptake of zinc by LNCaP and PC-3 cells

Cells were pretreated for 3 h with hormone or vehicle followed by incubation for 2 min in HBSS containing ^{65}Zn at a zinc concentration of 1 μg/ml. The uptake rate is ng of zinc/million cells/2 min. The values are the means (±S.E.) and percent increase *versus* control (no hormone).

Treatment	Zinc uptake rate	
	LNCaP cells	PC-3 cells
No hormone	28 (2)	9 (1)
Prolactin		
0.6 nM	35 (2) + 24%[a]	22 (2) + 144%[a]
3.0 nM	44 (2) + 57%[a]	
6.0 nM	47 (2) + 69%[a]	
Testosterone		
0.1 nM	40 (2) + 43%[a]	10 (1)
1.0 nM	53 (3) + 88%[a]	

[a] Statistically different *versus* control.

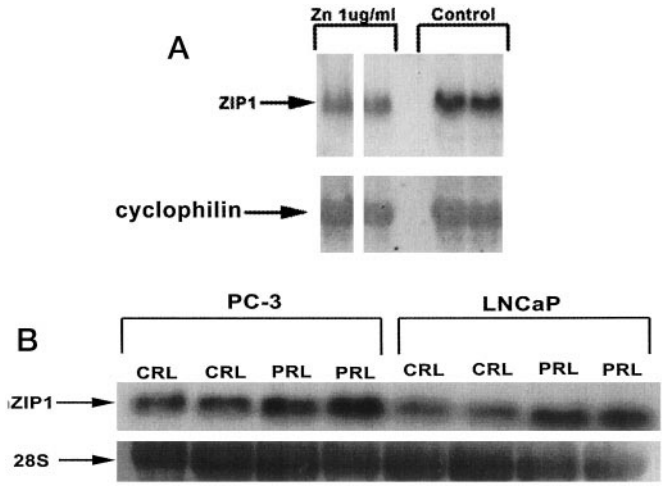

FIG. 5. **Effects of prolactin and zinc on the expression of hZIP1.** *Panel A*, down-regulation of hZIP1 by zinc. PC-3 cells were grown in medium supplemented with 10^{-9} M prolactin to increase the expression of hZIP1. The cells were then transferred to prolactin-free culture medium containing 1 μg/ml zinc or no zinc (control) and incubated for 24 h. The cells were then collected, washed, and probed for hZIP1 mRNA. *Panel B*, prolactin effect on the level of hZIP1 mRNA. LNCaP and PC-3 cells were incubated for 1 h in medium containing either 10^{-9} M prolactin or vehicle (control). After incubation, the cells were quickly washed, prepared for RNA extraction, and probed with ^{32}P-labeled hZIP1 cDNA clone. ^{32}P-Labeled cyclophilin cDNA probe and the 28 S RNA band are presented as gel-loading controls.

The studies described above provided compelling kinetic evidence for the existence of a zinc uptake transporter in prostate cells. The hormonal stimulation and its inhibition by actinomycin of zinc uptake led us to consider that the prostate cells likely expressed a rapid uptake transporter gene. A putative plasma membrane zinc transporter in humans has been identified based on the high homology to the *Arabidopsis* ZIP1 transporter (16, 19); therefore we proceeded to determine with the hZIP1 probe if this transporter might be expressed in the prostate cells. Fig. 5 demonstrates that both LNCaP and PC-3 cells exhibit expression of the putative hZIP1 transporter. Moreover, the level of the putative ZIP1 mRNA was increased by treatment of both cells with prolactin, indicative of hormonal regulation of a *hZIP1* transporter gene. A characteristic of some *ZIP* transporter genes is the down-regulation by exposure of cells to high zinc levels. Fig. 5 reveals that extended exposure of PC-3 cells to zinc resulted in a decrease in the level of ZIP1 mRNA indicative of a down-regulation of gene expression. The down-regulation by zinc adds to the evidence that we were probing the expression of a zinc uptake transporter.

DISCUSSION

The present studies demonstrate that the human malignant prostate cells LNCaP and PC-3 possess the ability to accumulate high zinc levels. The zinc levels of the prostate cells were significantly higher than non-prostate squamous carcinoma cells. This parallels the studies that demonstrated that rat prostate cells contained higher zinc levels than non-prostate (kidney or liver) cells (7). In addition fibroblast cell types reportedly contain about 0.4 fmol of zinc/cell (2). Estimates of the cell content of zinc in LNCaP cells are minimally (cells grown in "zinc-free" medium) about 1.0 fmol/cell, and in the presence of medium supplemented with 1 μg/ml zinc, about 2.0 fmol/cell. When treated with prolactin or testosterone, the zinc content is increased another 2–5-fold. Clearly, these cells accumulate much higher zinc levels than non-prostate cells. Moreover, in other unpublished studies we have demonstrated that when exposed to extracellular zinc, the accumulation of zinc by PC-3 cells results in the inhibition of their ability to oxidize citrate thereby causing in citrate accumulation. This demonstrates that the accumulated zinc includes a mobile reactive component that enters the mitochondria and inhibits *m*-aconitase activity (8). To protect against the toxic effects of zinc, mammalian cells generally employ defensive mechanisms that prevent the accumulation of mobile reactive zinc (3). In contrast, and consistent with their unique function, prostate secretory epithelial cells employ mechanisms that facilitate the uptke and accumulation of zinc.

The present studies demonstrate that prolactin and testosterone at physiological concentrations regulate zinc uptake and accumulation in LNCaP and PC-3 cells. Studies of zinc and citrate relationships with rat prostate lobes revealed that prostate epithelial cells can be characterized as the following types: V (ventral prostate), L (lateral prostate), and D (dorsal prostate) (3, 7). An important distinguishing characteristic is the response of the cells to prolactin and testosterone in the regulation of zinc accumulation. Zinc accumulation is decreased by both hormones in ventral prostate cells, increased by both hormones in lateral prostate cells, and unaffected by either hormone in dorsal prostate and non-prostate cells. Consequently, it became important to establish which cell type(s) exists in human prostate. The present studies reveal that both LNCaP and PC-3 cells exhibit the characteristics of lateral prostate cells. This is significant because rat lateral prostate is homologous to the lateral lobes of the peripheral zone of the human prostate. This region of the prostate is the dominant region for the origin of prostate malignancy from which the LNCaP and PC-3 cells were originally derived; therefore a linkage does exist between these cell lines and rat lateral prostate cells. However a most important relationship is that naturally occurring malignant prostate cells have lost the ability to accumulate zinc, whereas the malignant cell lines exhibit the capability of accumulating zinc. It will be essential to establish the reason why *in situ* malignant prostate cells do not accumulate zinc, which will lead to an understanding of the pathogenesis of prostate cancer.

The present studies establish that a rapid zinc uptake transport mechanism is associated with zinc accumulation by these cells and that this transport mechanism is regulated by prolactin and by testosterone. The initial kinetic studies indicate that the rapid transport would be highly effective under the conditions reflective of circulating levels of zinc. The most likely expectation is that prostate epithelial cells possess a plasma membrane zinc transporter that permits the rapid uptake of zinc from circulation. This would be similar to the unique aspartate transporter that is involved in the accumulation of high cellular levels of aspartate in prostate cells (21–

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23). We are now proceeding with extensive kinetic studies to establish the characteristics of this zinc transport process.

The fact that actinomycin completely abolished the stimulatory effects of both hormones on zinc uptake by LNCaP and PC-3 cells and that androgen receptor was required for testosterone stimulation of zinc uptake provided initial evidence that gene regulation is involved. Moreover, this is consistent with the hormonal regulation of the aspartate transporter and other citrate-related metabolic genes in prostate epithelial cells (4); therefore it seemed most plausible to pursue the possibility that the kinetic demonstration of the rapid uptake of zinc in the prostate cells might be the result of expression of a plasma membrane-associated zinc uptake transporter. However, little information is currently available regarding the expression of zinc uptake transporters in mammalian cells. The expression and operation of zinc transporters that export zinc out of cells and which sequester intracellular zinc into organelles have been identified in some mammalian cells (2, 24). In contrast, there has been no established identification of any gene expression associated with plasma membrane zinc uptake transporters in mammalian cells. Zinc uptake transporters have been identified in plants, yeast, and other organisms that comprise the homologous *ZIP* family of transporter genes (16–19). Eide and colleagues (16, 19) preliminarily identified two putative transporter genes in humans which are homologous to the ZIP family of zinc uptake transporters, one of which exhibits striking homology to *Arabidopsis* ZIP1.[2] The present studies showed that the human homolog, hZIP1, is expressed in LNCaP and PC-3 cells and that the expression is hormonally regulated. This is consistent with the hormonal regulation of the cellular accumulation of zinc and the rapid zinc uptake in these cells. The present results also demonstrate that the expression of *hZIP1* is down-regulated by exposure of cells to zinc, and this is characteristic of the zinc regulation of *ZIP1* and other zinc uptake transporters. The *Arabidopsis* ZIP1 has been characterized as a plasma membrane-associated zinc uptake transporter and as a rapid zinc uptake transporter that leads to the cellular accumulation of zinc (19). Such characteristics are surprisingly similar to the zinc uptake characteristics of LNCaP and PC-3 cells identified in this study. The striking similarities between the kinetic characteristics and hormonal regulation of zinc uptake in the prostate cells and the ZIP transporter provide compelling evidence for the expression and operation of a ZIP1 homolog (hZIP1) in prostate cells. This supports the contention (16, 19) that *ZIP* family zinc transporter genes are expressed in human cells; however, we cannot yet represent conclusively that the putative hZIP1 transporter is responsible for the rapid uptake transport process that has been identified kinetically in the prostate cells. It is conceivable that more than one zinc uptake transporter, as is the case for yeast and plant cells (16–19), might be involved in the uptake and accumulation of zinc in prostate epithelial cells. The results of the present study now provide the basis for further studies of the kinetic and genetic characterization of the zinc transport mechanism(s) in normal and malignant prostate cells, the mechanism of prolactin and testosterone regulation of zinc transport, the relationship of zinc accumulation in the pathogenesis and progression of prostate malignancy; they also provide a model system to study the mechanisms of zinc transport in mammalian cells in general.

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[2] D. Eide, personal communication.

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