Intestinal Crypt Stem Cells Possess High Levels of Cytoskeletal-associated Phosphotyrosine-containing Proteins and Tyrosine Kinase Activity Relative to Differentiated Enterocytes

David R. Burgess, Wanping Jiang, Salim Mamajiwalla, and William Kinsey

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, Florida 33101

Abstract. Growth and differentiation of stem cells is thought to be regulated by growth factors and responding protein tyrosine kinase activities. Comparing mitotic stem cells from the adult intestinal epithelium, isolated from the crypts of Lieberkuhn, with isolated differentiated absorbtive cells we find major differences in the levels of phosphotyrosine-containing proteins. Crypt stem cells possess two major phosphotyrosine-containing polypeptides of 36 and 17 kD which have greater than 15 times more phosphotyrosine than that present in the polypeptides of differentiated enterocytes. Tyrosine kinase activity and similar phosphotyrosine-containing proteins are associated with the Triton cytoskeleton. Moreover, crypt tyrosine kinase(s) is active in vitro in phosphorylating similar cytoskeleton-associated substrates. These results suggest that cytoskeleton-associated phosphotyrosine kinase(s) and their substrates may play a role in growth and differentiation of adult intestinal epithelial cells.

T HE regulation of cell growth during development and differentiation is poorly understood but must include fine control over cell division. Some hints as to the regulatory proteins involved in growth control have come from recent analysis of viral transforming gene products and their normal cell homologues (Bishop, 1985; Hunter and Cooper, 1985; Adamson, 1987). The unregulated expression of these viral oncogenes, such as *src*, leads to uncontrolled cell growth suggesting that one function of the cellular protooncogenes is to play some role in growth control. Many of these genes code for protein tyrosine kinases. Tyrosine kinase activity has also been found to be a property of the membrane receptors for several growth factors and polypeptide hormones, including EGF, PDGF, insulin, and insulin-like growth factor, reinforcing the hypothesis that this class of protein kinases plays an important role in growth control (Frackelton et al., 1984; Ek and Heldin, 1984; Kasuga et al., 1982; Saski et al., 1985).

Protein tyrosine kinases and their substrates are often localized to regions of the plasma membrane enriched in the actin cytoskeleton (Landreth et al., 1985; Takata and Singer, 1988; Glenney and Zokas, 1989). Moreover, there is a correlation between transformation and the association of the transforming protein of Rous sarcoma virus, pp60^{src}, with the cytoskeleton (Hamaguchi and Hanafusa, 1987). Recently, several studies have shown that tyrosine kinase activity is high in many normal embryonic tissues, including the intestinal epithelium, during periods of rapid proliferation (Maher and Pasquale, 1988; Takata and Singer, 1988).

The adult intestinal epithelium is a constantly differentiating system with a rapid turnover of cells (Weiser et al., 1986;

Gordon, 1989). This simple epithelium is generated by a dividing stem cell population residing in the crypts of Lieberkuhn. In the adult, cells cease division while still in the crypts and differentiate as they migrate out of the crypts and reside in the basal one-third of the villi. After 2–3 d, the differentiated cells die and are extruded from the tip of the villus. Studies of the cells along the crypt to tip axis reveal a gradient of cells of differing states of differentiation exhibiting gradients of gene expression, as evidenced by the biochemical and morphological characteristics of the brush border (Gordon, 1989).

In the present work we use an antibody against phosphotyrosine to identify proteins that act as substrates for protein tyrosine kinases in cells isolated from different regions of the adult intestinal epithelium characterized by different degrees of differentiation. Western blot analysis demonstrates that undifferentiated crypt cells possess two predominant phosphotyrosine-containing proteins of 17 and 36 kD. The crypt cell population, containing all of the proliferating cells, exhibits a much higher level of phosphotyrosine in these proteins than was detected in the differentiated cells. Phosphotyrosine-containing forms of proteins of the same molecular mass are exclusively associated with the cytoskeleton and are phosphorylated exclusively on tyrosine in vitro. Our results demonstrate that the 36- and 17-kD polypeptides and the protein tyrosine kinase(s) which phosphorylate them are associated with the cytoskeleton. These findings suggest that one or more cytoskeletal-associated tyrosine kinases may play a role in growth control in this actively dividing adult stem cell population.

Methods and Materials

Cell Isolation

The proximal loop of chicken duodena was excised, slit open, thoroughly rinsed in ice cold saline, and stirred in phosphate-buffered sucrose solution at room temperature to release intact epithelial cells from the submucosa (Matsudaira and Burgess, 1979). Fractions containing cells sequentially isolated from the tip and basal third of the villus and from the crypt were obtained by a modification of the method of Weiser (1973) and Breimer et al. (1981). Briefly, pieces 1–2-cm long were stirred in a beaker for a designated length of time and transferred to a new beaker for the next time period. Cell fractions were taken after 11 (tip cells), 16, 29 (basal third cells), 39, and 64 (crypt cells) min. The purity of cell fractions was assayed by several means including determination of alkaline phosphatase by the method of Weiser (1973), and light microscopy.

Cytoskeletons were prepared by gently shaking cells for 30 min in 0.1% Triton X-100 in 75 mM KCl, 0.1 mM $MgCl_2$, 5 mM EGTA, 10 mM Tris, pH 6.9, containing 0.2 mM PMSF, 1 mg/liter aprotinin, and 1 mg/liter SBTI at room temperature. Cells were then washed by low speed centrifugation and resuspended in the above buffer lacking Triton.

Immunoprecipitation and Immunoblot Analysis

The methods for purification and characterization of the antibodies against phosphotyrosine have been described in detail elsewhere (Peaucellier et al., 1988). For immunoprecipitation experiments, crypt cytoskeletons were phosphorylated in vitro by incubation in a phosphorylation buffer containing 10 mM Hepes, 10 mM $MnCl_2$, 10 μM Na_3VO_4, 10 μg/ml Aprotinin, and 0.15% NP-40. The reaction was started by addition of $[\gamma\text{-}^{32}P]ATP$ (435 Ci/mmol) to a final concentration of 3.0 μM and the samples were incubated at 25°C for 2 min. The ^{32}P-labeled proteins were solubilized in an immunoprecipitation buffer containing 150 mM NaCl, 50 mM Tris, 10 mM EDTA, 10 mM NaF, 10 μM Na_3VO_4, 5 mM PMSF, 1% Triton X-100, and 0.1 m/ml Aprotinin (Sigma Chemical Co., St. Louis, MO) and centrifuged at 100,000 g for 30 min at 4°C. The solubilized proteins were then incubated with affinity-purified antiphosphotyrosine antibody (0.3 μg/ml) at 4°C for 4 h after which 25 μl of protein A–Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) was added for each 0.1 μg of antibody. After 1 h, the immune complexes were collected by centrifugation, washed twice with immunoprecipitation buffer, once with 50 mM Tris, pH 7.5, solubilized in SDS gel sample buffer, and heated at 90°C for 5 min. The phosphoproteins were then analyzed by SDS micro slab PAGE, electroblotted onto Immobilon (Millipore/Continental Water Systems, Bedford, MA) membranes, and detected by autoradiography. For phosphoamino acid analysis, individual bands were cut out and hydrolyzed in 6 N HCl containing 50 μg each of phosphoserine, phosphothreonine, and phosphotyrosine at 110°C for 2 h. The hydrolysate was dried under vacuum and the phosphoamino acids were resolved by thin layer electrophoresis at pH 3.5.

Samples for Western immunoblot analysis were electrophoresed on SDS micro slab polyacrylamide gels and electrophoretically transferred to 0.45 μM Immobilon sheets. The sheets were then blocked in 150 mM NaCl, 50 mM Tris, pH 7.5, 0.2% NP-40, 1% gelatin, and 0.1% BSA for 12 h, after which they were incubated in blocking solution containing the affinity-purified antibody (0.3 μg/ml) for 4 h. After several washes in blocking buffer, the blots were incubated with ^{125}I-protein A 56 μCi/μg (ICN K & K Laboratories Inc., Plainview, NJ) at a concentration of 1 μCi/ml for 1 h. The blots were then washed in blocking buffer and dried.

Results

Characterization of Cell Fractions

The purity of each cell fraction was assayed in every preparation by phase-contrast microscopy which showed that the tip and crypt fractions were >95% pure (Fig. 1). The columnar tip cells possessed well-differentiated brush borders and were released in single cells to small sheets of cells (Fig. 1 A). The crypt fraction consisted of intact sacks of cells with the lumen internal, as in the intact tissue (Fig. 1 C). Crypt cells were cuboidal and were lacking in any distinct brush border. The mid fraction consisted of a few crypts mixed in
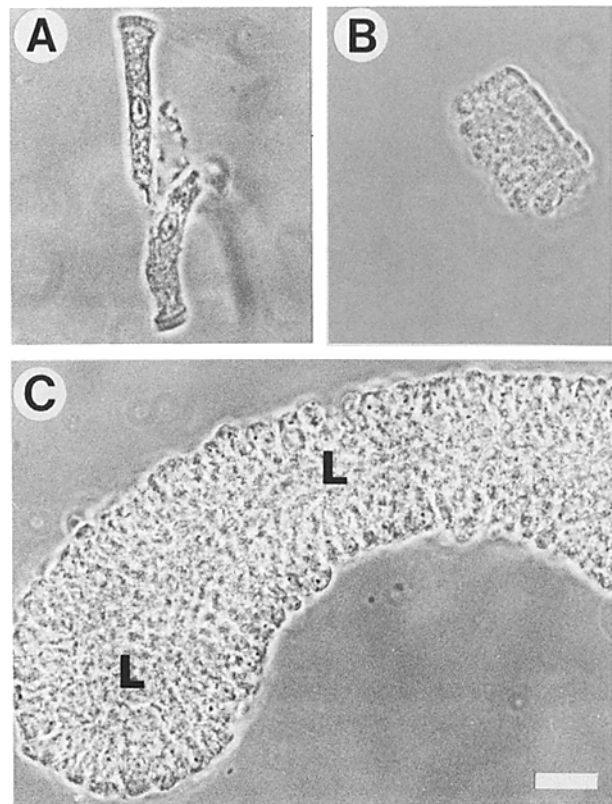

Figure 1. Phase-contrast micrographs of the isolated cell fractions. (*A*) Isolated tip cells are released as single cells to small groups of cells; the cells are tall, columnar with distinct brush borders. (*B*) The mid fraction is composed exclusively of sheets of cells; the cells are columnar, although shorter than tip cells and have distinct brush borders whose microvilli appear shorter than those of tip cells. (*C*) The crypt fraction is composed exclusively of intact sacks of short, cuboidal cells whose lumen (*L*) is internal. Bar, 20 μM.

with a majority of sheets of cells (Fig. 1 *B*). These sheets were composed of columnar cells, although not as tall as tip cells, which possessed brush borders with distinctly shorter microvilli than mature enterocytes. Using a brush border enzyme as a marker of the state of differentiation (Weiser, 1986), analysis of alkaline phosphatase activity from two separate cell preparations revealed that the tip cell fraction possessed 10-fold higher activity than did the crypt cells and about 4-fold higher than the mid cell fraction (tip = 24.27 U/mg; mid = 7.92 U/mg; crypt = 2.35 U/mg).

Phosphotyrosyl Proteins in Intact Cells

Samples of intact cells (equal amounts of protein) isolated from each region of the intestinal epithelium were run on 12% SDS-PAGE, electroblotted onto Immobilon, and probed for phosphotyrosyl-containing proteins with the antibody against phosphotyrosine. These Western blots revealed two major phosphotyrosyl-containing proteins with relative mobilities of 36 and 17 kD (Fig. 2, *left*). In addition, two polypeptides of ~50 and 45 kD were preferentially phosphorylated in crypt cells, and one very high molecular weight polypeptide was phosphorylated in crypt and basal villus cells, although at much lower levels than the 36- and 17-kD polypeptides. Interestingly, one protein of ~62-kD was found
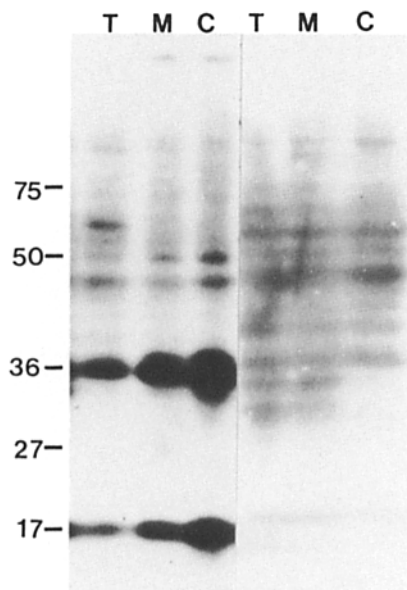
Table I. Relative Phosphotyrosine Content of 17- and 36-kD Proteins

		Tip	Mid	Crypt
17-kD experiments	a	1	1.1	6.5
	b	1	1.2	15.5
	c	1	7.3	38.3
36-kD experiments	a	1	4.1	9.5
	b	1	0.3	16.4
	c	1	10.3	29.1
	d	1	1.7	5.8

Cells isolated from the intestinal epithelium were analyzed by SDS–PAGE and blotted onto nitrocellulose. The blots were treated with the antibody to phosphotyrosine or with the antibody together with 5 mM phosphotyrosine as a competitive inhibitor. Antibody binding was detected by incubation with ^{125}I-protein A followed by autoradiography. The phosphotyrosine content of the 17-kD ($n = 3$) and 36-kD ($n = 4$) proteins was estimated by scanning the autoradiographs and subtracting the values obtained in the presence of 5 mM phosphotyrosine from those obtained with the antibody alone. The values obtained from the tip fraction were set at 1.0 and the values from the other cell fractions are expressed relative to this value.

Figure 2. Detection of phosphotyrosine-containing proteins in the intestinal epithelium. Cells (100-μg protein) isolated from the tip of the villus (T), the basal third of the villus (M), and the crypt (C) were analyzed by SDS–PAGE and the proteins were blotted onto nitrocellulose. Phosphotyrosine-containing proteins were detected by incubating the blot with the antibody to phosphotyrosine. In one group of samples (right) 5 mM phosphotyrosine was included with the antibody to act as a competitive inhibitor. After washing, the bound antibody was detected with ^{125}I-protein A and autoradiography. Two prominent bands of $\sim$36 and 17 kD bound the antibody as seen in the left panel. However, phosphotyrosine completely inhibited antibody binding to these proteins (right) indicating that the antibody bound through an interaction with phosphotyrosine.

to be phosphorylated on a tyrosine only in the villus tip cells. The specificity of the antibody recognizing phosphotyrosine was confirmed by adding 5 mM free phosphotyrosine as a competitive inhibitor while the blot was incubating with the antibody (Fig. 2, right).

As seen in Fig. 2, a significant difference existed in the amount of phosphotyrosine-containing 36- and 17-kD polypeptides in cells of differing differentiated states. Significantly higher levels of phosphotyrosine associated with the 36- and 17-kD polypeptides were present in undifferentiated crypt cells than in either villus base or tip cells. Similarly, cells of the villus base possessed more phosphotyrosine in the 36- and 17-kD polypeptides than did villus tip cells. Quantitation of the relative levels of phosphotyrosine in the 36- and 17-kD polypeptides, by scanning autoradiographs from four separate experiments, revealed that crypt stem cells had on average 15 times more phosphotyrosine in 36-kD and an average of 20 times more phosphotyrosine in 17-kD than villus tip cells (Table I).

Cytoskeletal Association of Phosphotyrosyl Proteins

To determine whether these phosphotyrosine-containing proteins were associated with the cytoskeleton, equal amounts of protein from the Triton-soluble and -insoluble (cytoskeleton) fractions of isolated villus tip, villus basal third, and crypt cells were probed on a Western blot with the antibody

against phosphotyrosine (Fig. 3). Our work and that by other investigators has shown that this cytoskeleton fractionation results in release of soluble proteins, dissolution of the plasma and internal membranes, and retention of the actin cytoskeleton, including the brush border cytoskeleton (Burgess, 1987). All phosphotyrosine-containing polypeptides were found associated with the detergent-insoluble cytoskeletal fraction. As in intact cells, the 36- and 17-kD polypeptides were the predominate phosphotyrosine-containing species. Several higher molecular mass phosphotyrosine-containing polypeptides were also detected. Moreover, as was found for intact cells, the level of phosphotyrosine-containing polypeptides was significantly higher in crypt cell cytoskeletons than in those of the basal third and tip of the villus.

In Vitro Tyrosine Phosphorylation of Crypt Cytoskeletons

Several attempts to detect in vivo phosphorylation of proteins in intact pieces of intestine by metabolic labeling proved unsuccessful because of poor uptake of phosphate, so we under-
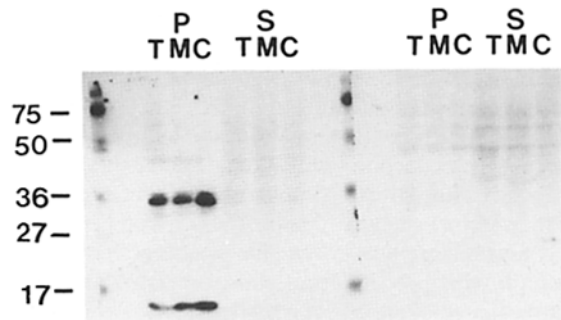

Figure 3. Detection of phosphotyrosine-containing proteins in the cytoskeletal fraction of intestinal cells. Villus tip (T), basal third (M), and crypt cells (C) were isolated, the 0.1% Triton X-100–soluble (S) and –insoluble (P) proteins precipitated with 10% TCA, and analyzed by SDS-PAGE. Phosphotyrosine containing proteins were detected by Western blot analysis using the antibody to phosphotyrosine as in Fig. 2. Samples on the right panel were incubated with antibody + 5 mM phosphotyrosine as a competitive inhibitor.
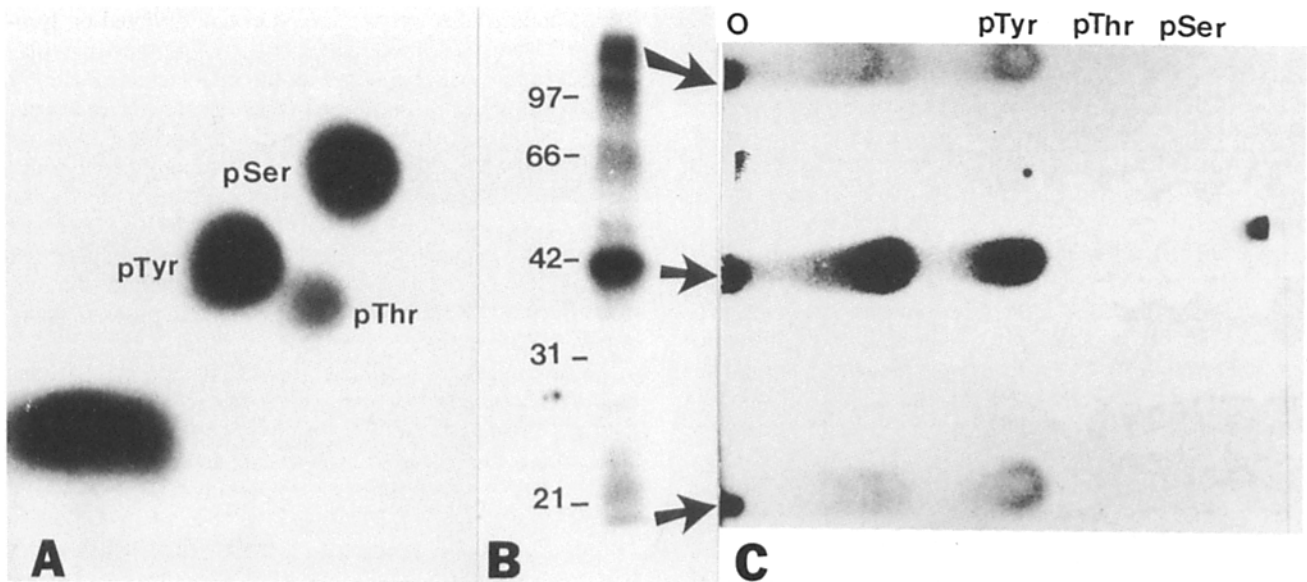
Burgess et al. Cytoskeletal Tyrosine Kinase in Crypt Stem Cells

2141

Figure 4. (A) In vitro phosphorylation of the cytoskeleton preparation from intestinal crypt cells. A sample (200 µg protein) of the detergent-insoluble cytoskeleton pellet was incubated in phosphorylation buffer containing 10 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 10 µM Na_3VO_4, and 50 µM γ^{32}P-ATP (62.5 Ci/mmol) for 2 min at 25°C. The reaction was stopped by addition of 10% TCA and the precipitated proteins were washed with TCA, extracted with ether, and hydrolyzed in 6 N HCl at 110°C for 2 h. The samples were dried under vacuum, analyzed by two-dimensional high voltage electrophoresis at pH 1.9 (bottom to top) and at pH 3.5 (left to right), and then dried and subjected to autoradiography. The position of authentic standards included in the sample was detected by staining with ninhydrin. After autoradiography, the radioactive spots were cut out and counted in a scintillation counter: 43% of the counts were in phosphotyrosine, 41% in phosphoserine, and 16% in phosphothreonine. (B) Crypt cells were extracted with Triton X-100 and the detergent-insoluble cytoskeleton pellet was allowed to undergo phosphorylation in vitro with radioactive ATP as in A. The phosphorylated proteins were solubilized in immunoprecipitation buffer and incubated with the antibody to phosphotyrosine. The immune complexes were recovered by incubation with protein A–Sepharose and analyzed by SDS–PAGE. The phosphoproteins immunoprecipitated by this technique were detected by autoradiography of the gel. (C) Phosphorylated proteins immunoprecipitated as in A were resolved by SDS–PAGE and blotted onto Immobilon. The three predominant radioactive bands including the high molecular mass bands near the the top of the gel (97–180 kD), the 36-kD band, and the 17-kD band, were localized by autoradiography, cut out, and hydrolyzed in 6 N HCl, and the phosphoamino acids analyzed by thin layer electrophoresis at pH 3.5. The position of standard phosphoamino acids was determined by staining with ninhydrin. O, origin.

took in vitro phosphorylation experiments with isolated crypt cell cytoskeletons. Isolated crypt cytoskeletons were incubated for a period of time with a phosphorylation buffer containing γ^{32}P-ATP. A fraction of the total in vitro–phosphorylated cytoskeletal proteins were hydrolyzed in HCl and analyzed by two-dimensional high voltage electrophoresis for phosphoamino acid content (Fig. 4 A). The autoradiograph of this analysis revealed a substantial amount of phosphotyrosine and phosphoserine with a small amount of phosphothreonine. The regions containing individual phosphoamino acids were cut out and counted in a scintillation counter. Of the total radioactivity incorporated into phosphoamino acids, 43% was in phosphotyrosine, 41 in phosphoserine, and 16% in phosphothreonine, clearly demonstrating that the cytoskeleton is highly enriched in tyrosine kinase activity.

To ascertain what proteins were phosphorylated on tyrosine in vitro, the radiolabeled cytoskeletal proteins were dissolved in immunoprecipitation buffer and the phosphotyrosine-containing proteins precipitated with antibody against phosphotyrosine. Analysis of the subsequent immunoprecipitated phosphorylated polypeptides by SDS–PAGE revealed two major phosphotyrosine-containing polypeptides of 17 and 36 kD (Fig. 4 B). However, the 36- and 17-kD polypeptides, while heavily phosphorylated, were not the only major phosphorylated polypeptides. Several other higher molecu-

lar mass polypeptides, ranging in molecular mass from 97 to 180 kD, were phosphorylated on tyrosine in vitro.

The phosphoamino acid composition of the ^{32}P-labeled proteins detected by analysis of immunoprecipitates, as in Fig. 4 B, was determined by blotting the gel onto Immobilon and cutting out the radioactive bands for partial acid hydrolysis and thin layer electrophoresis (Fig. 4 C). This analysis chemically confirmed that all phosphorylated polypeptides immunoprecipitated with the antibody against phosphotyrosine contained phosphotyrosine. Moreover, the 36-, 17-, and 97–180 kD (high molecular mass) polypeptides were phosphorylated only on tyrosine and not on serine or threonine.

Discussion

In this study we have demonstrated that undifferentiated mitotic stem cells in the intestinal epithelium are characterized by much higher levels of phosphotyrosine-containing proteins than are the differentiated cells located along the intestinal villus. Two cytoskeleton-associated polypeptides of 36 and 17 kD are the predominant protein tyrosine kinase substrates in the crypt cells. Moreover, tyrosine protein kinase activity is also associated with the cytoskeleton and is active in vitro.

Recent studies of developing embryonic chicken intestine

(Maher and Pasquale, 1988; Takata and Singer, 1988) demonstrate that the immature, mitotically active intestinal tissue contains high levels of phosphotyrosine-containing proteins relative to that found in the mature intestine. By immunofluorescence, no phosphotyrosyl-containing proteins were detectable in adult differentiated villus absorbtive cells (Takata and Singer, 1988); unfortunately, no mention was made as to whether crypts were examined. We have been unable to localize phosphotyrosine-containing proteins in crypt tissue. Inspection of the Western blot results from Maher and Pasquale (1988) reveal that a phosphotyrosine-containing protein of ~36 kD was also present in embryonic intestines from d 8 through 16, a period during which the entire epithelium is mitotically active (Overton and Shoup, 1964). The phosphotyrosine content appears to be reduced beginning at d 17 at which time other studies have shown that mitoses begin to be restricted to developing crypts as villi begin to elongate (Overton and Shoup, 1964). Since it appears that entire intestinal segments, including muscle, were used by Maher and Pasquale (1988) it is likely that the inability to detect phosphotyrosyl-containing proteins in the adult was due to sensitivity in picking up a signal from a small amount of crypt tissue surrounded by a very large mass of submucosa. Thus, one difference between the embryonic and the adult epithelium is that mitoses are restricted to crypts in the adult (Potten and Loeffler, 1987; Gordon, 1989), whereas mitoses occur throughout the epithelium of the previllus ridges or early villi in the embryo. Our results combined with those studies identifying and localizing phosphotyrosyl-containing proteins therefore show that the highest levels of tyrosine kinase activity are correlated with mitotically active stem cells in both the embryonic and the adult intestinal epithelium.

Two brush border cytoskeleton-associated proteins have been identified whose function, unlike many of the other brush border cytoskeletal proteins, is unknown but whose properties are unusual in normal cells. One of these proteins, termed ezrin or p81 (Gould et al., 1986), is associated with the microvillus actin bundle (Bretscher, 1983), while the other, called calpactin I (or p36), is part of the terminal web cytoskeleton (Gerke and Weber, 1984). Both of these proteins, along with vinculin, are in vitro substrates for protein tyrosine kinases (Gould et al., 1986; Gerke and Weber, 1984; Bretscher, 1989). Calpactin I appears to be present in a higher concentration in differentiated villus cells, as is likely for ezrin, than in crypt cells (Greenberg et al., 1984). Calpactin's in vitro binding to actin or spectrin, and phosphorylation by pp60^{src}, is enhanced by calcium (albeit in unphysiologically high levels). Calpactin also binds to phospholipids; these findings are consistent with it being part of the membrane skeleton of nonmuscle cells (Glenney, 1985; 1986; Glenney et al., 1987). However, nothing is known about the in vivo function of this protein. It is possible that the phosphotyrosine-containing 36-kD polypeptide associated with the crypt cytoskeleton is calpactin I, especially since it has been shown that calpactin I associates with the Triton cytoskeleton prepared in the absence of calcium (Gould et al., 1986; Zokas and Glenney, 1987). We have recently found that the calpactins are phosphorylated in vitro in crypt cytoskeletons (Burgess, D. R., and W. Kinsey, unpublished observations). The identities of the 17-kD and higher molecular mass phosphotyrosine-containing polypeptides are unknown at this time.

Our findings that crypt proteins phosphorylated on tyrosine are cytoskeleton-associated suggest that they may play some role in mediating some motile cellular event such as mitosis, cytokinesis, migration in these stem cells, or assembly of the brush border. Interestingly, Bretscher (1989) has recently shown that tyrosine phosphorylation of ezrin (p81) in A-431 cells correlates with major reorganizations of the actin cytoskeleton. Exactly how phosphorylation may affect these properties in vivo is unknown at this time. Further work will be necessary to determine the reason for the dramatic decrease in level of phosphotyrosine in proteins as enterocytes differentiate.

We have recently found that crypt cells possess 5-10 times higher in vitro protein tyrosine kinase activity of pp60^{c-src} than cells from the villus tip (Cartwright, C., W. Eckhart, S. Mamajiwalla, and D. R. Burgess, manuscript in preparation). Since we find that one phosphotyrosine-containing protein in crypt cells is likely p36, calpactin I, our identification of pp60^{c-src} kinase activity is consistent with earlier reports that intestinal p36 is a good substrate in vitro for pp60^{v-src} (Glenney and Tack, 1985). On the other hand, since many cellular tyrosine kinases are plasma membrane–associated receptors for growth factors, our results do not rule out a role for growth factor receptor tyrosine kinase activity. Recently, several reports demonstrated that EGF promotes maturation, including the stimulation of DNA synthesis and the precocious appearance of specific digestive enzymes, of the intestinal epithelium in fetal and neonatal rodent intestines (for review see Lebenthal and Leung, 1987).

References

Adamson, E. D. 1987. Oncogenes in development. *Development.* 99:449–471.

Bishop, J. M. 1985. Viral oncogenes. *Cell.* 42:23–38.

Breimer, M. E., G. C. Hansson, K.-A. Karlsson, and H. Leffler. 1981. Glycosphingolipids and the differentiation of intestinal epithelium. *Exp. Cell Res.* 135:1–13.

Bretscher, A. 1983. Purification of an 80,000-dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells. *J. Cell Biol.* 97:425–532.

Bretscher, A. 1989. Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in A-431 cells by epidermal growth factor. *J. Cell Biol.* 108:921–930.

Burgess, D. R. 1987. The brush border: a model for structure biochemistry, motility, and assembly of the cytoskeleton. *In* Advances in Cell Biology. Vol. 1. K. R. Miller, editor. JAI Press Ltd., London. 31–58.

Ek, B., and C. H. Heldin. 1984. Use of an antiserum against phosphotyrosine for the identification of phosphorylated components in human fibroblasts stimulated by platelet-derived growth factor. *J. Biol. Chem.* 259:11145–11152.

Frackelton, A. R., P. M. Tremble, and L. T. Williams. 1984. Evidence for the platelet-derived growth factor–stimulated tyrosine phosphorylation of the platelet-derived growth factor receptor in vivo. *J. Biol. Chem.* 259:7909–7915.

Gerke, V., and K. Weber. 1984. Identity of p36K phosphorylation upon Rous sarcoma virus transformation with a protein purified from brush borders: calcium-dependent binding to non-erythroid spectrin and F-actin. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:227–233.

Glenney, J. R., Jr. 1985. Phosphorylation of p36 in vitro with pp60^{src}: regulation by Ca^{2+} and phospholipid. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 192:79–82.

Glenney, J. R., Jr. 1986. Two related but distinct forms of the 36,000 Mr tyro-

sine kinase substrate (calpactins) which interact with phospholipid and actin in a Ca^{2+}-dependent manner. *Proc. Natl. Acad. Sci. USA.* 83:4258–4262.

Glenney, J. R., Jr., and B. F. Tack. 1985. Amino-terminal sequence of p36 and associated p10: identification of the site of tyrosine phosphorylation and homology with S-100. *Proc. Natl. Acad. Sci. USA.* 82:7884–7888.

Glenney, J. R., Jr., and L. Zokas. 1989. Novel tyrosine kinase substrates from Rous sarcoma virus transformed cells are present in the membrane skeleton. *J. Cell Biol.* 108:2401–2408.

Glenney, J. R., Jr., B. F. Tack, and M. A. Powell. 1987. Calpactins: two distinct Ca^{++}-regulated phospholipid- and actin-binding proteins isolated from lung and placenta. *J. Cell Biol.* 104:503–511.

Gordon, J. I. 1989. Intestinal epithelial differentiation: new insights from chimeric and transgenic mice. *J. Cell Biol.* 108:1187–1194.

Gould, K. L., J. A. Cooper, A. Bretscher, and T. Hunter. 1986. The protein-tyrosine kinase substrate, p81, is homologous to a chicken microvillar core protein. *J. Cell Biol.* 106:660–669.

Greenberg, M. E., R. Brackenbury, and G. M. Edelman. 1984. Changes in distribution of the 34-kdalton tyrosine kinase substrate during differentiation and maturation of chicken tissues. *J. Cell Biol.* 98:473–486.

Hamaguchi, M., and H. Hanafusa. 1987. Association of pp60^{src} with Triton X-100-resistant cellular structure correlates with morphological transformation. *Proc. Natl. Acad. Sci. USA.* 84:2312–2316.

Hunter, T., and J. A. Cooper. 1985. Protein-tyrosine kinases. *Annu. Rev. Biochem.* 54:897–930.

Kasuga, M., F. A. Karlsson, and C. R. Kahn. 1982. Insulin stimulates the phosphorylation of the 95,000 dalton subunit of its own receptor. *Science (Wash. DC).* 215:185–187.

Landreth, G. E., L. K. Williams, and G. D. Reiser. 1985. Association of the epidermal growth factor receptor kinase with the detergent-insoluble cytoskeleton of A431 cells. *J. Cell Biol.* 101:1341–1350.

Lebenthal, E., and Y.-K. Leung. 1987. Epidermal growth factor (EGF) and the ontogeny of the gut. *J. Pediatr. Gastroenterol. Nutr.* 6:1–13.

Maher, P. A., and E. B. Pasquale. 1988. Tyrosine phosphorylated proteins in different tissues during chick embryo development. *J. Cell Biol.* 106:1747–1755.

Matsudaira, P. T., and D. R. Burgess. 1979. Identification and organization of the components in the isolated microvillus cytoskeleton. *J. Cell Biol.* 83:667–673.

Overton, J., and J. Shoup. 1964. Fine structure of cell surface specializations in the maturing duodenal mucosa of the chick. *J. Cell Biol.* 21:75–85.

Peaucellier, G., P. A. Veno, and W. H. Kinsey. 1988. Protein tyrosine phosphorylation in response to fertilization. *J. Biol. Chem.* 263:13806–13811.

Potten, C. S., and M. Loeffler. 1987. A comprehensive model of the crypts of the small intestine; the mouse provides insight into the mechanisms of cell migration and the proliferation heirarchy. *J. Theor. Biol.* 127:381–391.

Saski, N., R. W. Rees-Jones, Y. Zick, S. P. Nissley, and M. M. Rechler. 1985. Characterization of insulin-like growth factor I-stimulated tyrosine kinase activity associated with the B-subunit of type I insulin-like growth factor receptors of rat liver cells. *J. Biol. Chem.* 260:9793–9804.

Takata, K., and S. J. Singer. 1988. Phosphotyrosine-modified proteins are concentrated at the membranes of epithelial and endothelial cells during tissue development in chick embryos. *J. Cell Biol.* 106:1757–1764.

Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* 248:2536–2541.

Weiser, M. M., J. R. F. Walters, and J. R. Wilson. 1986. Intestinal cell membranes. *Int. Rev. Cytol.* 101:1–57.

Zokas, L., and J. R. Glenney, Jr. 1987. The calpactin light chain is tightly linked to the cytoskeletal form of calpactin I: studies using monoclonal antibodies to calpactin subunits. *J. Cell Biol.* 105:2111–2121.