2. S. C. Erwin and M. R. Pederson, *ibid.* **67**, 1610 (1991).
3. A. F. Hebard *et al.*, *Nature* **350**, 600 (1991).
4. M. J. Rosseinsky *et al.*, *Phys. Rev. Lett.* **66**, 2830 (1991).
5. K. Tanigaki *et al.*, *Nature* **352**, 222 (1991); S. P. Kelty, C.-C. Chen, C. M. Lieber, *ibid.*, p. 223.
6. P. J. Benning, J. L. Martins, J. H. Weaver, L. P. F. Chibante, R. E. Smalley, *Science* **252**, 1417 (1991).
7. G. Stollhoff, in preparation.
8. W. E. Pickett, H. Krakauer, R. E. Cohen, *Physica B* **165 & 166**, 1055 (1990).
9. P. W. Stephens *et al.*, *Nature* **351**, 632 (1991).
10. K. Holczer *et al.*, *Phys. Rev. Lett.* **67**, 271 (1991).
11. Y. J. Uemura *et al.*, *Nature* **352**, 605 (1991).
12. P. B. Allen, in *Modern Trends in the Theory of Condensed Matter: Proc. XVI Karpacz Winter School of Theoretical Physics*, A. Pekalski and J. Przystawa, Eds. (Springer-Verlag, New York, 1980), pp. 388–413.
13. J. L. Martins, N. Troullier, M. Schabel, in preparation.
14. F. C. Zhang, M. Ogata, T. M. Rice, in preparation.
15. Partial support is acknowledged from the Office of Naval Research. Calculations were performed on the IBM 3090 at the Cornell National Supercomputing Facility.

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Modulation of the Affinity of Integrin $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) by the Cytoplasmic Domain of α_{IIb}

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Intracellular signaling alters integrin adhesive functions in inflammation, immune responses, hemostasis, thrombosis, and retinal development. By truncating the cytoplasmic domain of α_{IIb}, the affinity of integrin $\alpha_{IIb}\beta_3$ for ligand was increased. Reconstitution with the cytoplasmic domain from integrin α_5 did not reverse the increased affinity. Thus, the cytoplasmic domain of the α subunit of GPIIb-IIIa controls ligand binding affinity, which suggests mechanisms for inside-out transmembrane signaling through integrins. These findings imply the existence of hitherto unappreciated hereditary and acquired thrombotic disorders in humans.

INTEGRIN-MEDIATED ADHESION participates in diverse biological processes such as development, inflammation, wound repair, and hemostasis (*1*). The cellular repertoire of integrins and the availability of adhesive ligands control these processes. In addition, integrin function is dynamically regulated by cells in response to developmental signals (*2*), cell-cell interactions (*3*), or soluble agonists (*4*). Integrin adhesive functions are controlled by their affinity for ligands. For example, $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) (*4*) or $\alpha_M\beta_2$ (Mac-1) (*5*) bind soluble fibrinogen (Fg) only after cellular stimulation. In $\alpha_{IIb}\beta_3$, this affinity change is due to alterations in the conformation of the receptor (*6*).

Intracellular signal transduction that involves G proteins, calcium, phospholipid metabolism, and kinases (7) is implicated in affinity modulation of integrins, but the link between these pathways and changes in receptor conformation has remained elusive. The putative cytoplasmic domains of α and β subunits are topographically accessible to intracellular signals and may therefore mediate these affinity changes. To test this possibility, we have examined the effect of truncations of the cytoplasmic domains of $\alpha_{IIb}\beta_3$ on its affinity state. This integrin was chosen because dynamic changes in its affinity (*4*) and ligand binding specificity (*8*) are readily assayed by use of Fg or an activation-specific monoclonal antibody (MAb), PAC1 (*9*), which binds to an epitope that is only revealed after activation.

Truncated α_{IIb} and β_3 subunits were generated by in vitro mutagenesis (*10*); the truncated α_{IIb} ($\alpha_{IIb}\Delta991$) retained two residues of the putative cytoplasmic domain, whereas the truncated β_3 ($\beta_3\Delta728$) retained six residues (Fig. 1A). Chinese hamster ovary (CHO) cell lines that expressed these constructs were analyzed by immunoprecipitation of surface-labeled cells (Fig. 1B) with site-specific antisera to the extracellular domain of α_{IIb} (amino acids 859 to 871) (*11*) and the cytoplasmic domains of α_{IIb} (amino acids 989 to 1008) and β_3 (amino acids 742 to 762) (Fig. 1B). The wild-type $\alpha_{IIb}\beta_3$ was immunoprecipitated with all three antisera. In contrast, $\alpha_{IIb}\beta_3\Delta728$ was immunoprecipitated with anti-α_{IIb}(859–871) and anti-α_{IIb}(989–1008), but not with anti-β_3(742–762). Conversely, $\alpha_{IIb}\Delta991\beta_3$ was precipitated by anti-α_{IIb}(859–871) and anti-β_3(742–762), but not by anti-α_{IIb}(989–1008). A double truncation, $\alpha_{IIb}\Delta991$–$\beta_3\Delta728$, was immunoprecipitated with anti-α_{IIb}(859–871), but not with the cytoplasmic domain antibodies.

The affinity state of $\alpha_{IIb}\beta_3$ in these cell lines was initially characterized by the binding of the MAb PAC1 (Fig. 2). This anti-$\alpha_{IIb}\beta_3$ interacts selectively with the active, not the inactive, receptor and presumably binds at or near the ligand binding pocket, because its interaction competes with typical $\alpha_{IIb}\beta_3$ ligands (*12*). The $\alpha_{IIb}\beta_3$ and $\alpha_{IIb}\beta_3\Delta728$ transfected cells did not bind PAC1 specifically unless they were activated

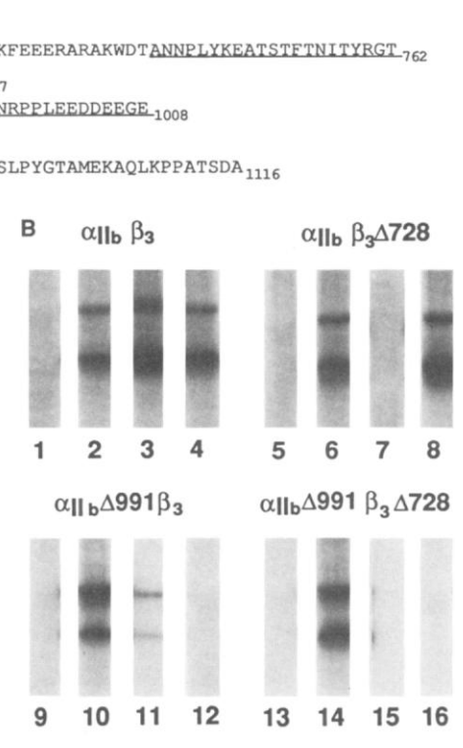

Fig. 1. $\alpha_{IIb}\beta_3$ truncation in CHO cells. (**A**) The partial transmembrane (bold) and complete cytoplasmic amino acid sequence for wild-type (WT) or variant α_{IIb} and β_3 constructs. The subscripts denote residue number, whereas the underlined sequences denote peptide immunogens for cytoplasmic domain–specific antisera. Single letter abbreviations for the amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**B**) Wild-type or truncated α_{IIb} and β_3 subunits were identified by immunoprecipitation from extracts of surface-labeled cells. Cells were labeled with ^{125}I and then lysed and immunoprecipitated as described (*6*) with polyclonal antisera to an extracellular portion of α_{IIb}(859–871) (*11*) and the cytoplasmic domains of α_{IIb}(989–1008) and β_3(742–762). Immunoprecipitates were resolved by SDS–6% polyacrylamide gels electrophoresis and analyzed by autoradiography. Lanes 1, 5, 9, and 13, preimmune serum; lanes 2, 6, 10, and 14, anti-α_{IIb}(859–871); lanes 3, 7, 11, and 15, anti-β_3(742–762); and lanes 4, 8, 12, and 16, anti-α_{IIb}(989–1008).

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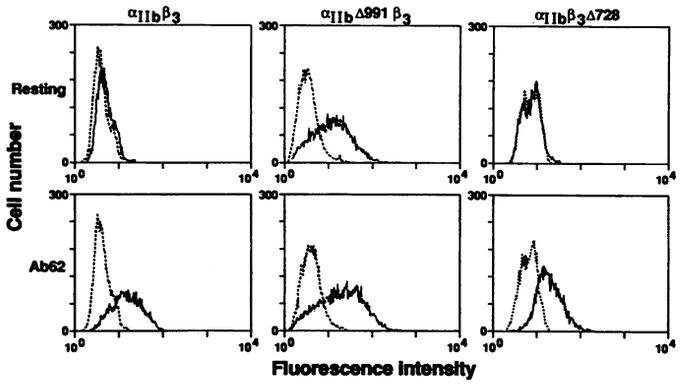

Fig. 2. CHO cells expressing truncated α_{IIb} bind PAC1 in the absence of activators. The binding of PAC1 to CHO cells expressing $\alpha_{IIb}\beta_3$, $\alpha_{IIb}\Delta991\beta_3$, or $\alpha_{IIb}\beta_3\Delta728$ was measured by flow cytometry. Cells were harvested, stained with PAC1 in the presence or absence of 10 mM GRGDSP or 6 μM MAb 62, and analyzed by flow cytometry as described (*6*). Histograms of PAC1 binding in the presence of GRGDSP (dotted line) have been superimposed upon histograms of binding in its absence (solid line).

Table 1. Specificity of Fg binding to $\alpha_{IIb}\Delta991\beta_3$. ^{125}I-Fg (200 nM) was incubated with CHO cells (2×10^8 cells/ml) bearing either the wild-type ($\alpha_{IIb}\beta_3$) or the α_{IIb} cytoplasmic domain truncated ($\alpha_{IIb}\Delta991\beta_3$) form of the receptor at 22°C for 30 min in the presence of EDTA (10 mM), an inhibitory MAb to $\alpha_{IIb}\beta_3$, 4F10 (2 nM) or no inhibitor. Activating MAb 62 (*6*) (6 μM) was added to the indicated reactions to verify the functional competence of wild-type $\alpha_{IIb}\beta_3$. After a 30-min incubation, bound Fg was measured (*6*). Data are expressed as Fg bound (molecules/cell) × 10^3 ± SE.

Inhibitor	$\alpha_{IIb}\Delta$ 991β_3	$\alpha_{IIb}\beta_3$	$\alpha_{IIb}\beta_3$ + mAb62
None	89.9 ± 6.9	16.6 ± 0.4	62.6 ± 2.3
EDTA	27.1 ± 2.3	15.9 ± 1.3	21.7 ± 0.8
4F10	16.2 ± 0.7	21 ± 0.6	33.2 ± 1.8

with MAb 62 (anti-LIBS2) (*6*). MAb 62 binds to the extracellular domain of β_3 and induces a change in $\alpha_{IIb}\beta_3$ that causes increased ligand binding of this integrin (*6*). In contrast, $\alpha_{IIb}\Delta991\beta_3$ bound PAC1 without MAb 62 addition, suggesting that it was constitutively active. PAC1 binding was specific, because it was blocked with 1 mM GRGDSP peptide (*12*).

The $\alpha_{IIb}\Delta991\beta_3$-bearing cells bound soluble ^{125}I-Fg without addition of MAb 62 (Table 1). Fibrinogen binding was inhibited with MAb to $\alpha_{IIb}\beta_3$, 4F10 (*13*) or EDTA. In contrast (*6*), wild-type $\alpha_{IIb}\beta_3$ did not detectably bind soluble Fg in the absence of an activator. Analysis of the equilibrium binding of soluble Fg to $\alpha_{IIb}\Delta991\beta_3$ by the LIGAND (*14*) program indicated a K_A ± SE of $1.25 \pm 0.13 \times 10^7\ M^{-1}$ (K_D = 80 nM), which is similar to the K_D of 110 nM that was reported for the activated wild-type receptor (*6*). Fibrinogen binding to $\alpha_{IIb}\Delta991\beta_3$ was divalent cation–dependent and inhibited by a MAb to $\alpha_{IIb}\beta_3$. Thus, the $\alpha_{IIb}\Delta991\beta_3$ is probably in the same affinity state as exogenously activated $\alpha_{IIb}\beta_3$. The $\alpha_{IIb}\Delta991\beta_3\Delta728$ mutant also bound soluble ^{125}I-Fg (K_A of $1.43 \times 10^7\ M^{-1} \pm 0.5 \times 10^7\ M^{-1}$) and PAC1 [mean fluorescence intensity (MFI) of 71.2; MFI in the presence of GRGDSP was 15.0] constitutively.

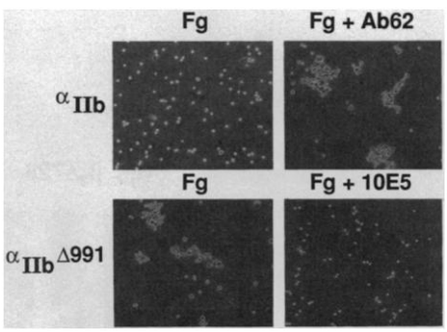

Fig. 3. Agonist-independent aggregation of cells bearing $\alpha_{IIb}\Delta991\beta_3$. Cells expressing the indicated integrin α subunit were harvested with trypsin and EDTA, washed, and resuspended in Tyrode's solution at 10^7 per milliliters (*15*). Cells (100 μl) were added to wells of a 24-well tissue culture plate in the presence or absence of 6 μM MAb 62 and subjected to gyrorotation (100 rpm for 30 min). Fibrinogen (1 μM) was added and aggregation was stopped after 20 min by addition of 2% paraformaldehyde (150 μl). An inhibitory anti-$\alpha_{IIb}\beta_3$ [MAb 10E5, 500 nM (*16*)] was added at the time of Fg addition to the $\alpha_{IIb}\Delta991\beta_3$ cells (right, lower panel).

Cells aggregate after Fg binding to activated $\alpha_{IIb}\beta_3$. The CHO cells that expressed recombinant $\alpha_{IIb}\beta_3$ exhibited Fg-dependent aggregation only after addition of activating antibody (*15*). In contrast, cells expressing $\alpha_{IIb}\Delta991$ aggregated upon Fg addition in the absence of activator (Fig. 3) and aggregation was blocked with MAb to $\alpha_{IIb}\beta_3$ (*16*).

Because of the unexpected properties of $\alpha_{IIb}\Delta991\beta_3$, we constructed $\alpha_{IIb}\alpha_5$, a chimeric integrin with the α_{IIb} extracellular and transmembrane domains and an α_5 cytoplasmic domain (Fig. 1). When expressed in CHO cells, $\alpha_{IIb}\alpha_5\beta_3$ was reactive with prototype anti-$\alpha_{IIb}\beta_3$ antibodies [10E5 (*16*), Tab (*17*), MAb15 (*18*)] and was constitutively active with respect to PAC1 binding (MFI of 165.5 and of 19.1 in the absence and presence, respectively, of GRGDSP, compared to MFI values of 19.4 and 18.5, respectively, for $\alpha_{IIb}\beta_3$ transfectants) and Fg binding (K_A ± SE of $2.1 \pm 0.6 \times 10^7\ M^{-1}$, K_D = 47 nM). Because the α_5 cytoplasmic domain is eight residues longer than that of α_{IIb}, the activation of $\alpha_{IIb}\Delta991\beta_3$ is the result of deletion of specific α_{IIb} sequences rather than truncation per se.

These results suggest that sequences in the cytoplasmic domain of α_{IIb} control the affinity state of $\alpha_{IIb}\beta_3$. These sequences may bind to an intracellular moiety to maintain the receptor in a low affinity conformation. Modification of this moiety during cell activation could disrupt its interaction with α_{IIb}, thereby inducing the high affinity state. Because CHO cells rather than human platelets were used, such repressors could have a wide distribution, yet it must be integrin-specific; the α_5 cytoplasmic–domain chimera did not reconstitute the low affinity state. Modification of affinity may also involve cell-specific mechanisms, because platelets but not CHO cells (*6*) modulate $\alpha_{IIb}\beta_3$ in response to agonists. The β_3 cytoplasmic tail might be the moiety recognized by α_{IIb}(991–1008). Disruption of these intramolecular interactions could then induce high affinity ligand binding. The failure of $\beta_3\Delta728$ to be constitutively active or to reduce the activation induced by $\alpha_{IIb}\Delta991$ seems inconsistent with this. Although other interactions involving the cytoplasmic domain of α_{IIb} can be envisioned, the determinants of integrin activation may be the nature of repressor-integrin interactions and whether such interactions can be modified.

Although β subunit–cytoplasmic sequences have been implicated in the events after ligand binding, such as focal contact formation (*19*) and adhesion to immobilized substrate (*19, 20*), no functional role has yet been established for α subunit–cytoplasmic domains. Our results suggest that these sequences are involved in affinity modulation and this is supported by the association of α_6 phosphorylation with increased macrophage adhesion to laminin (*21*). Our data also suggest that the cell type-specific, developmentally regulated alternative splicing of the cytoplasmic domains of α_3 and α_6 (*22*) may thus alter ligand binding affinities of $\alpha_3\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$.

Based on these findings, spontaneous mutations that truncate the cytoplasmic domain of α_{IIb} should be pathogenic in man. The α_{IIb} subunit is apparently platelet-specific (*23*), and platelet aggregation is normal when only 50% of the $\alpha_{IIb}\beta_3$ is functional (*24*), indicating that such mutations would be dominant. In a hereditary form, the

predicted disease could have features of congenital thrombocytopenia and a thrombotic tendency due to spontaneous platelet aggregation. Because some myeloproliferative syndromes are due to clonal proliferation of stem cells that affects platelets (*25*), somatic mutations that truncate α_{IIb} could result in an acquired thrombotic tendency. Somatic mutations that affect other integrin α subunit–cytoplasmic domains in malignant cells may also influence their invasive and metastatic properties.

REFERENCES AND NOTES

1. R. O. Hynes, *Cell* **48**, 549 (1987); E. Ruoslahti and M. D. Pierschbacher, *Science* **238**, 491 (1987).
2. K. M. Neugebauer and L. F. Reichardt, *Nature* **350**, 68 (1991).
3. M. L. Dustin and T. A. Springer, *ibid.* **341**, 619 (1989).
4. J. S. Bennett and G. Vilaire, *J. Clin. Invest.* **64**, 1393 (1979); G. A. Marguerie, T. S. Edgington, E. F. Plow, *J. Biol. Chem.* **255**, 154 (1980).
5. D. C. Altieri, R. Bader, P. M. Mannucci, T. S. Edgington, *J. Cell Biol.* **107**, 1893 (1988).
6. T. E. O'Toole *et al.*, *Cell Regulation* **1**, 883 (1990).
7. S. J. Shattil and L. F. Brass, *J. Biol. Chem.* **262**, 992 (1987); L. F. Brass, M. Laposata, H. S. Banga, S. E. Rittenhous, *ibid.* **261**, 16838 (1986).
8. E. F. Plow and M. H. Ginsberg, *ibid.* **256**, 9477 (1981).
9. S. J. Shattil, J. A. Hoxie, M. Cunningham, L. F. Brass, *ibid.* **260**, 11107 (1985).
10. The $\beta_3\Delta 728$ construct was made by Eco RI digestion of BS3a (*26*), isolation of the coding fragment, filling in overhangs with T4 polymerase (*27*), and ligation to an Xba I linker with a termination codon in all three reading frames. This fragment was cut with Xba I, its ends polished with mung bean nuclease, ligated to a Bst XI linker, digested with Bst XI, and ligated to Bst XI–digested CDM8 (*26*). The $\alpha_{IIb}\Delta 991$ construct was made by generating a Sal I site in BS2b (*26*) at bases 3061 to 3066 (*28*). After digestion with Sal I and isolation of the fragment, the ends were partially filled in with T4 polymerase and polished with mung bean nuclease (*27*). This fragment was ligated to an Xba I linker containing a termination codon in all three reading frames, digested with Xba I, and ligated to Xba I–cut and dephosphorylated CDM8. The $\alpha_{IIb}\alpha_5$ chimera was generated in two steps. A 600-bp Hind III–Xba I fragment that contained the cytoplasmic sequence of α_5 was isolated from α_5 cDNA and ligated to Hind III + Xba I–digested CDM8 generating $CD\alpha_5 3'$. Then, a Hind III site was introduced into α_{IIb} at bases 3058 to 3063. After Hind III digestion, the coding fragment was isolated and ligated to Hind III–digested and dephosphorylated $CD\alpha_5 3'$.
11. J. C. Loftus, E. F. Plow, L. Jennings, M. H. Ginsberg, *J. Biol. Chem.* **263**, 11025 (1988).
12. R. Taub *et al.*, *ibid.* **264**, 259 (1989).
13. V. L. Woods, Jr., L. E. Wolff, D. M. Keller, *ibid.* **261**, 15242 (1986).
14. P. J. Munson and D. Rodbard, *Anal. Biochem.* **107**, 220 (1980).
15. M. M. Frojmovic, T. E. O'Toole, E. F. Plow, J. C. Loftus, M. H. Ginsberg, *Blood*, **78**, 369 (1991).
16. B. S. Coller, E. I. Peerschke, L. E. Scudder, C. A. Sullivan, *J. Clin. Invest.* **72**, 325 (1983).
17. R. P. McEver, E. M. Bennett, M. N. Martin, *J. Biol. Chem.* **258**, 5269 (1983).
18. A. L. Frelinger III *et al.*, *ibid.* **265**, 6346 (1990).
19. J. Solowska, J-L. Guan, E. E. Marcantonio, J. E. Trevithick, C. A. Buck, R. O. Hynes, *J. Cell Biol.* **109**, 853 (1989).
20. M. L. Hibbs, H. Xu, S. A. Stacker, T. A. Springer, *Science* **251**, 1611 (1991).
21. L. M. Shaw, J. M. Messier, A. M. Mercurio, *J. Cell Biol.* **110**, 2167 (1990).
22. R. N. Tamura, H. M. Cooper, G. Collo, V. Quaranta, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
23. S. Suzuki *et al.*, *J. Biol. Chem.* **262**, 14080 (1987).
24. T. J. Kunicki, D. Pidard, J-P. Cazenave, A. T. Nurden, J. P. Caen, *J. Clin. Invest.* **67**, 717 (1981).
25. R. Munker and H. P. Koeffler, in *Hematology*, R. Hoffman, E. J. Benz, S. J. Shattil, B. Furie, H. J. Cohen, Eds. (Churchill Livingstone, New York, 1991), pp. 817–834.
26. T. E. O'Toole *et al.*, *Blood* **74**, 14 (1989).
27. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
28. T. A. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 488 (1985).

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Mechanism for Regulating Cell Surface Distribution of Na^+, K^+-ATPase in Polarized Epithelial Cells

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Restriction of sodium, potassium adenosine triphosphatase (Na^+, K^+-ATPase) to either the apical or basal-lateral membrane domain of polarized epithelial cells is fundamental to vectorial ion and solute transport in many tissues and organs. A restricted membrane distribution of Na^+, K^+-ATPase in Madin-Darby canine kidney (MDCK) epithelial cells was found experimentally to be generated by preferential retention of active enzyme in the basal-lateral membrane domain and selective inactivation and loss from the apical membrane domain, rather than by vectorial targeting of newly synthesized protein from the Golgi complex to the basal-lateral membrane domain. These results show how different distributions of the same subunits of Na^+, K^+-ATPase may be generated in normal polarized epithelia and in disease states.

SODIUM, POTASSIUM ADENOSINE TRIphosphatase (Na^+, K^+-ATPase) is a plasma membrane protein in animal cells that functions in the maintenance of homeostasis. In many cell types, Na^+, K^+-ATPase is uniformly distributed over the cell surface. However, in polarized epithelial cells the cell surface distribution of the same subunits of Na^+, K^+-ATPase may be restricted to either the apical [for example, choroid plexus (*1*), retinal pigmented epithelium (*2*)] or basal-lateral [for example, intestine, kidney (*3*)] membrane domain. Localization of Na^+, K^+-ATPase to either domain establishes a transepithelial Na^+ gradient that drives vectorial transport of ions and solutes between the two compartments separated by the epithelium. In some epithelial diseases, such as polycystic kidney disease (*4*) and ischemia (*5*), the cell surface polarity of Na^+, K^+-ATPase appears to be partially or completely reversed.

Several mechanisms have been proposed to explain how cell surface distributions of membrane proteins are regulated in polarized epithelial cells. These include sorting of newly synthesized proteins in the Golgi complex and vectorial delivery to the appropriate membrane domain, rerouting of protein from one membrane to the other, and interaction of sorted proteins with the membrane-cytoskeleton (*6, 7*). To investigate mechanisms that regulate the cell surface distribution of Na^+, K^+-ATPase, we analyzed stages during differentiation of renal epithelial cells when unpolarized precursor cells are converted to polarized epithelial cells (*6*). During this time, the initially uniform distribution of Na^+, K^+-ATPase becomes restricted to the basal-lateral membrane. We have used MDCK cells as an in vitro model of polarized renal epithelia (*6, 7*), in which induction of Ca^{2+}-dependent cell-cell contacts through E-cadherin results in the development of structural and functional polarity in stages similar to those that occur during renal epithelial development in vivo (*6, 8*).

Ca^{2+}-dependent cell-cell contacts were induced in confluent monolayers of MDCK cells (*9*), and the distribution of Na^+, K^+-ATPase was visualized by indirect immunofluoresence with laser-scanning confocal microscopy (Fig. 1A). For as long as 48 hours after cell-cell contact, we detected staining of the α subunit of Na^+, K^+-ATPase at both the apical membrane and at

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