of sensitivity, at the 12.5th and the 17.5th day after conception, were observed (*22*). For irradiation, a Westinghouse 150 Industrial X-ray Machine was used (130 kilovolt peak–x-rays, delivered by a self-rectifying tube, inherent filtration 1.65-mm aluminum). A current of 8 mA was used to obtain an intensity at 40 cm of 24 ± 2 rad/min. The mice were exposed in individual sterile polypropylene-polyethylene containers resting on a 24-cm-diameter steel turntable. The mice were rotated to ensure a more accurate average value of the irradiated field. The delivered dose was measured for each irradiation with a Victoreen C-r 570 meter.

26. These doses were read from the radiometer that was put into the irradiation chamber next to the mouse container.
27. The experiments with single hairs were done similarly to experiments described for a single cell–based assay of the "mouse spot test" (*16*). The six unirradiated offspring were from three independent litters. Six mice were irradiated with 100 cGy at day 17.5 after conception, and 12 offspring were used. The skin of the mice was prepared as described by Searle and Stephenson (*16*). The animals were killed by decapitation 6 or 7 days after birth. Blood was allowed to drain away, and each mouse was dissected to remove the skin. The skins were then treated with 10% formalin for at least 24 hours, washed in distilled water, transferred to 70% ethanol for 24 hours, transferred to 90% ethanol for 24 hours, and incubated in two changes of absolute ethanol for 12 hours each. The skins were then treated with a 1:1 benzyl:ethyl alcohol mixture for 12 hours and with pure benzyl alcohol for 12 hours. Any adherent muscle tissue was removed with blunt-end forceps to leave the skin as thin as possible. The skin was divided into 12 dorsolateral segments, and each segment was mounted in Canada balsam with the dorsal surface uppermost on a microscope slide under a cover slip. The slides were then left to dry on a hot plate at 40°C. Each slide was examined in a bright field under 100-fold magnification and the follicles and hair counted.
28. We thank the members of the Schiestl laboratory, as well as J. B. Little and L. Harrison for discussions and comments on the manuscript. Supported in part by a "Reproductive Hazard in the Workplace, Home, Community and Environment Research grant" 15-FY93-0025 from the March of Dimes Birth Defect Foundation to R.H.S., by grant ES06593 from the National Institutes of Health to R.H.S., and by a fellowship from the Islamic Development Bank to F.K.

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Association of Insulin Receptor Substrate–1 with Integrins

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Insulin stimulation was found to promote association of the $\alpha_v\beta_3$ integrin (a vitronectin receptor) with insulin receptor substrate–1 (IRS-1), an intracellular protein that mediates insulin signaling by binding other signaling molecules, including growth factor receptor–bound protein 2 (Grb2) and phosphatidylinositol-3′ kinase. Insulin-treated cells expressing the $\alpha_v\beta_3$ integrin showed 2.5 times more DNA synthesis when plated on vitronectin than on other substrates, whereas cells expressing another vitronectin receptor, $\alpha_v\beta_5$, did not show this difference. The association between integrin and IRS-1 may be a mechanism for the synergistic action of growth factor and extracellular matrix receptors.

Adhesion of cells to extracellular matrix (ECM) is mainly mediated by the integrin family of cell surface receptors (*1*) and is a prerequisite for cell proliferation and survival (*2*). The pathways for integrin signaling and growth factor signaling are thought to be mechanistically linked because cell adhesion to ECM is required for cells to respond to certain growth factors (*3*) and integrin-mediated cell adhesion and motility can be modulated by growth factors (*4*). Furthermore, growth factor treatment can disrupt focal adhesions, the presumed sites of integrin-mediated signaling (*5*).

To investigate whether integrins associate with molecules involved in growth factor signaling, we studied Rat-1 fibroblasts that had been stably transfected with DNA encoding the human insulin receptor (HIRcB cells) (*6*). We immunoprecipitated integrins from these cells and searched for coprecipitated tyrosine-phosphorylated proteins by immunoblotting with antibody to phosphotyrosine (anti-pTyr). Two polyclonal antibodies against the $\alpha_v\beta_3$ integrin and a polyclonal antibody against the platelet $\alpha_{IIb}\beta_3$ integrin coprecipitated a 185-kD phosphorylated protein (p185) from insulin-stimulated but not quiescent HIRcB cells (Fig. 1A, lanes 1, 3, 4, and 5). Two other polyclonal antibodies to $\alpha_v\beta_3$ did not coprecipitate p185, possibly because they disrupt the integrin-p185 association (*7*). No coprecipitation of p185 occurred with polyclonal antibodies against the $\alpha_5\beta_1$ integrin (a fibronectin receptor) (Fig. 1A, lane 6) (*8*) or with antibodies against unrelated proteins (Fig. 1A, lane 7). Similar results were obtained with human HepG2 hepatoma cells (*9*). When anti-$\alpha_v\beta_3$ immunocomplexes were dissociated in SDS and reprecipitated with the same antibodies, p185 was not detectable in the reprecipitated receptor complex (*10*), suggesting that antibodies to integrins do not directly cross-react with p185 under these conditions (Fig. 1A, lane 8).

Isolation of HIRcB cell integrins on an affinity column confirmed the association of p185 with integrins. The phosphorylated p185 copurified with detergent extracts from insulin-treated cells on GRGDSPK-Sepharose, a known ligand for $\alpha_v\beta_3$ and other integrins (*11*), but not on Sepharose linked to a control peptide, GRGESPK (Fig. 1B).

Insulin receptor substrate–1 (IRS-1) is the major target protein phosphorylated on tyrosine by ligand-activated insulin receptor and has an apparent molecular mass of 185 kD on SDS-polyacrylamide gels (*12*). Immunoblot analysis with an antiserum against the NH_2-terminus of IRS-1 (*13*) demonstrated the presence of a reactive band in anti-$\alpha_v\beta_3$ immunocomplexes from insulin-stimulated but not quiescent HIRcB cells (Fig. 1C). Similar results were obtained with two other polyclonal antibodies to IRS-1, one raised against the COOH-terminus of IRS-1 and the other against recombinant IRS-1 produced in insect cells (*14*). The antiserum against the NH_2-terminus of IRS-1, however, was used throughout this study, as was anti-$\alpha_v\beta_3$ 237, unless otherwise indicated. Dissociation of the anti-$\alpha_v\beta_3$ immunocomplexes from insulin-treated cells and subsequent reprecipitation with anti–IRS-1 yielded a band corresponding to IRS-1 (Fig. 1D). The fact that the bands produced by anti-$\alpha_v\beta_3$ and anti–IRS-1 (Fig. 1D, lanes 1 and 2) were of about equal intensity, and the failure of the phosphotyrosine antibody to immunoprecipitate any phosphotyrosine-containing proteins from the dissociated anti-$\alpha_v\beta_3$ complexes after they had been depleted of IRS-1 (Fig. 1D, lane 3), showed that IRS-1 is a major portion of p185 associated with the vitronectin receptor. About 5 to 8% of IRS-1 associated with the vitronectin receptor, and maximal association was reached after 3 min of insulin stimulation (*15*). The insulin receptor was not detectable in the integrin immunocomplex (*14*).

Tyrosine-phosphorylated IRS-1 links insulin receptor activation to downstream intracellular signaling pathways through its association with proteins containing the Src homology 2 (SH2) domain. These proteins include the Ras guanine nucleotide–releasing complex Grb2-Sos, phosphatidylinositol-3′ kinase (PI-3 kinase), the phosphotyrosine phosphatase Syp, and the adaptor protein Nck (*12*). We found that at least two of these proteins, Grb2 and PI-3 kinase, were precipitated by anti-$\alpha_v\beta_3$ from the insulin-stimulated HIRcB cells (Fig. 2). Whether other IRS-1–binding proteins are also associated with integrins after insulin stimulation remains to be tested.

To investigate the physiological effects of the integrin–IRS-1 association, we studied FG human pancreatic carcinoma cells which do not express $\alpha_v\beta_3$, but rather use

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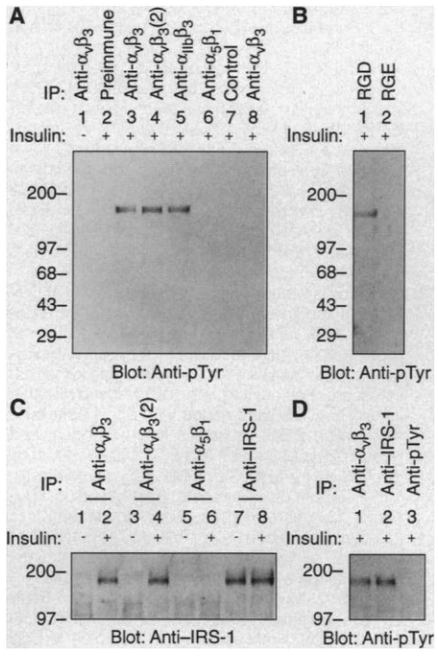

Fig. 1. Association of p185 (IRS-1) with integrins. (**A**) Lysates of quiescent (−) or insulin-stimulated (+) HIRcB cells were immunoprecipitated with the following antibodies: anti-$\alpha_v\beta_3$ 237 (lanes 1 and 3), preimmune serum for 237 (lane 2), anti-$\alpha_v\beta_3$ 1343 [anti-$\alpha_v\beta_3$(2)] (lane 4), anti-$\alpha_{IIb}\beta_3$ (lane 5), anti-$\alpha_5\beta_1$ (lane 6), and an unrelated rabbit polyclonal antibody (lane 7) (*23*). Separate stained gels showed that the antibodies immunoprecipitated the appropriate integrins. In lane 8, anti-$\alpha_v\beta_3$ 237 immunocomplexes from insulin-treated cells were dissociated and reprecipitated with the same antibody (*24*). The immunocomplexes were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with a monoclonal anti-pTyr and ^{125}I-labeled antibody to mouse immunoglobulin G (anti–mouse IgG) (*25*). (**B**) Lysates of insulin-stimulated HIRcB cells (*26*) were isolated on GRGDSPK-Sepharose (RGD, lane 1) or GRGESPK-Sepharose (RGE, lane 2), and eluates were analyzed by anti-pTyr immunoblotting. (**C**) Lysates of quiescent or insulin-stimulated HIRcB cells were immunoprecipitated with the indicated antibodies. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with anti–IRS-1 followed by chemiluminescence detection with anti–rabbit IgG (ECL, Amersham). The amount of protein loaded in lanes 1 to 6 was five times that in lanes 7 and 8. (**D**) Anti-$\alpha_v\beta_3$ immunocomplexes from insulin-treated cells (lane 1) were dissociated as in (A). The supernatant was subjected to three rounds of reprecipitation with the anti–IRS-1 (lane 2), and the supernatant cleared of IRS-1 was immunoprecipitated with anti-pTyr antibody (lane 3). The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with anti-pTyr. Molecular sizes are indicated to the left of gels in kilodaltons.

integrin $\alpha_v\beta_5$ as their vitronectin receptor (*16*). IRS-1 became tyrosine-phosphorylated in insulin-stimulated FG-C cells (Fig. 3A, lane 2) (*17*), but it did not associate with the $\alpha_v\beta_5$ integrin (Fig. 3A, lanes 3 and 4) (*18*). In contrast, integrin association of IRS-1 was found in FG-B cells that had been stably transfected with a β_3 integrin complementary DNA (cDNA) and thus express the $\alpha_v\beta_3$ integrin (Fig. 3A, lanes 5 and 7) (*17*). No association of the β_1 integrins with IRS-1 was seen (Fig. 3A, lane 8). The FG-B cells responded to insulin with about a 2.5-fold higher level of DNA synthesis when plated on vitronectin (an $\alpha_v\beta_3$-$\alpha_v\beta_5$ integrin ligand) compared with collagen (a β_1 integrin ligand) (Fig. 3B). The $\alpha_v\beta_3$-negative FG-C cells showed the same response to insulin whether plated on vitronectin or collagen. A correlation between $\alpha_v\beta_3$ expression and increased insulin responsiveness on vitronectin was also found in a study of three $\alpha_v\beta_3$-expressing cell lines and three lines expressing only $\alpha_v\beta_5$. By using these cell lines, we could also show that attachment to laminin does not increase insulin responsiveness (*19*).

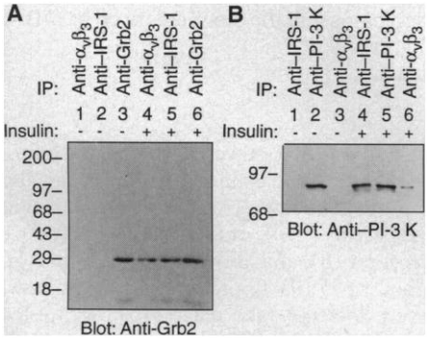

Fig. 2. Association of Grb2 (**A**) and PI-3 kinase (**B**) with integrins. Lysates of quiescent (−) or insulin-stimulated (+) HIRcB cells were immunoprecipitated with the indicated antibodies (*25*). The amount of protein loaded in (A) in the anti-$\alpha_v\beta_3$ lanes was 5 times that in the anti–IRS-1 lanes, and 10 times that in the anti-Grb2 lanes. In (B), equal amounts of proteins were loaded in the lanes. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted. Immunoreactive proteins were detected with anti–mouse IgG or anti–rabbit IgG, respectively, and chemiluminescence.

Our results demonstrate a physical association between an integrin and a protein that functions in growth factor signaling. Although these experiments cannot distinguish a preexisting association from one that occurs after the cells have been extracted, the cell proliferation data suggest that the $\alpha_v\beta_3$ integrin, presumably through its interaction with IRS-1, modulates cellular responses to insulin in a ligand-dependent manner. The integrin association may increase the level of phosphorylated IRS-1 at the plasma membrane, where some of the targets for the IRS-1–associated signaling molecules are localized, and thereby enhance insulin-mediated signaling.

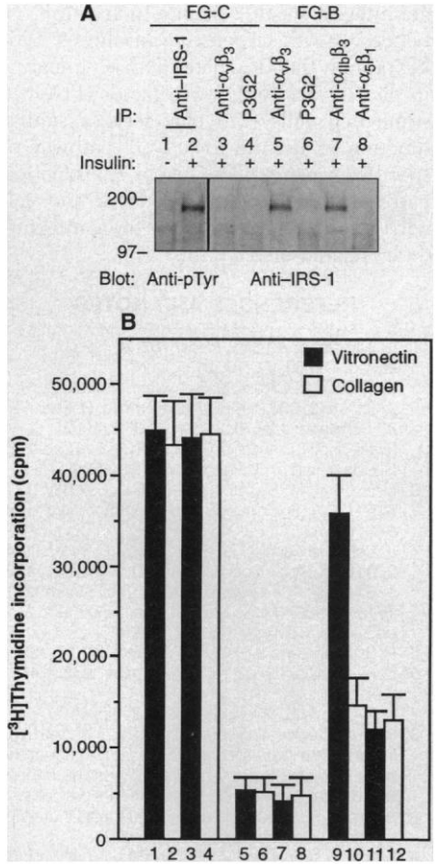

Fig. 3. Integrin–IRS-1 association and DNA synthesis in insulin-stimulated human pancreatic carcinoma cells. (**A**) Lysates of quiescent (−) or insulin-stimulated (+) cells were immunoprecipitated with the indicated antibodies (*22*). Immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with anti-pTyr (lanes 1 and 2) or anti–IRS-1 (lanes 3 to 7). (**B**) Incorporation of [^{3}H]thymidine into DNA. FG-B (samples 1, 2, 5, 6, 9, and 10) and FG-C cells (samples 3, 4, 7, 8, 11, and 12) were transferred to 24-well plates (1×10^5 cells per well) that had been coated with vitronectin or type I collagen. Cells were incubated for 36 hours in DMEM containing either 10% FCS (samples 1 to 4) or 0.1% BSA (samples 5 to 12), followed by addition of buffer (samples 5 to 8) or 100 nM insulin (samples 9 to 12) (*27*). After 15 hours, the cells were pulse-labeled with [^{3}H]thymidine (1 μCi/ml) (specific activity 6.7 Ci/mmol, DuPont NEN) for 3 hours, and thymidine incorporation into DNA was determined by trichloroacetic acid precipitation and liquid scintillation counting. Error bars indicate the standard error of three determinations.

Unlike the activation of focal adhesion kinase, which seems to be mediated by a number of integrins (*5*, *20*), the association with IRS-1 appears to be limited to the $\alpha_v\beta_3$ integrin, at least in the cell lines examined so far. IRS-1 could therefore medi-

ate integrin-specific signals, such as the enhanced growth response to insulin. A 190-kD protein that associates with α_v integrins in platelet-derived growth factor (PDGF)–stimulated cells (*21*) may serve a similar function in linking the PDGF pathway to specific integrins. The signal transduction pathways of other growth factor and cell adhesion receptors are likely to be integrated by related mechanisms.

REFERENCES AND NOTES

1. E. Ruoslahti, *J. Clin. Invest.* **87**, 1 (1991); R. O. Hynes, *Cell* **69**, 11 (1992).
2. M. Stoker, C. O'Neill, S. Berryman, V. Waxman, *Int. J. Cancer* **3**, 683 (1968); J. E. Meredith, B. Fazeli, M. A. Schwartz, *Mol. Biol. Cell* **4**, 953 (1993); S. M. Frisch and H. Francis, *J. Cell Biol.* **124**, 619 (1994); E. Ruoslahti and J. C. Reed, *Cell* **77**, 1 (1994).
3. L. E. Lilien, M. Sendtner, M. Raff, *J. Cell Biol.* **111**, 635 (1990); D. Schubert and H. Kimura, *ibid.* **114**, 841 (1991).
4. J. Schlessinger and B. Geiger, *Exp. Cell Res.* **134**, 273 (1981); B. J. Bockus and C. D. Stiles, *ibid.* **153**, 186 (1984); T. Kadowaki *et al.*, *J. Biol. Chem.* **261**, 16141 (1986); M. L. Stracke *et al.*, *Biochem. Biophys. Res. Commun.* **153**, 1076 (1988).
5. R. L. Juliano and S. Haskill, *J. Cell Biol.* **3**, 577 (1993).
6. D. A. McClain *et al.*, *J. Biol. Chem.* **262**, 14663 (1987).
7. K. Vuori and E. Ruoslahti, data not shown.
8. Immunoprecipitation showed that the $\alpha_3\beta_1$ and $\alpha_5\beta_1$ integrins are expressed in HIRcB cells at about the same level as $\alpha_v\beta_3$ (K. Vuori and E. Ruoslahti, unpublished data). Because all β_1 integrins are precipitated by anti-$\alpha_5\beta_1$, this negative result suggests that other β_1 integrins do not associate with p185.
9. Monoclonal antibody (mAb) LM609 against the $\alpha_v\beta_3$ integrin complex and mAb 147 against the α_v integrin subunit coprecipitated p185, whereas mAb P4C10 against the β_1 subunit and mAb P1D6 against the α_5 subunit did not (*22*).
10. SDS treatment did not alter the amount of $\alpha_v\beta_3$ integrin precipitated by any of the anti-$\alpha_v\beta_3$, a result demonstrated for anti-$\alpha_v\beta_3$ 237 in (*21*).
11. E. Ruoslahti and M. D. Pierschbacher, *Science* **238**, 491 (1987). Abbreviations for the amino acid residues are D, Asp; E, Glu; G, Gly; K, Lys; P, Pro; R, Arg; and S, Ser.
12. M. F. White and C. R. Kahn, *J. Biol. Chem.* **269**, 1 (1994); S. R. Keller and G. E. Lienhard, *Trends Cell Biol.* **4**, 115 (1994).
13. D. W. Rose, A. R. Saltiel, M. Majumdar, S. J. Decker, J. M. Olefsky, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 797 (1994).
14. K. Vuori and E. Ruoslahti, data not shown. Polyclonal anti–IRS-1 and antibodies to the insulin receptor were from Upstate Biotechnology (Lake Placid, NY).
15. Quantitation of anti–IRS-1 immunoblots was performed with an Ambis radioanalytic imaging system.
16. D. A. Cheresh, J. W. Smith, H. M. Cooper, V. Quaranta, *Cell* **57**, 59 (1989).
17. FG-C and FG-B are FG sublines transfected with pcDNAINeo and with β_3 cDNA in pcDNAINeo, respectively. The FG-C cells express the $\alpha_v\beta_5$ integrin and β_1 integrins, and the FG-B cells [D. I. Leavesley, G. D. Ferguson, E. A. Wayner, D. A. Cheresh, *J. Cell Biol.* **117**, 1101 (1992)] additionally express the $\alpha_v\beta_3$ integrin at a level of about 60% that of β_1 (K. Vuori and E. Ruoslahti, unpublished data).
18. We confirmed that the polyclonal antibodies to $\alpha_v\beta_3$ precipitated the $\alpha_v\beta_5$ integrin, as previously shown (*17*, *21*).
19. K. Vuori and E. Ruoslahti, unpublished data. The $\alpha_v\beta_3$-expressing lines were HIRcB, NIH 3T3, and NRK cells, and the lines expressing $\alpha_v\beta_5$ but not $\alpha_v\beta_3$ were HT29 colon carcinoma, UCLA-P3 lung adenocarcinoma, and Panc-1 pancreatic carcinoma cells. The $\alpha_v\beta_3$ associated with IRS-1 in all of the $\alpha_v\beta_3$-expressing cells after insulin stimulation. All of the cells attached equally well to vitronectin. All of the cell lines attached to laminin, collagen, or both, and this was inhibited by antibodies to β_1.
20. M. D. Schaller and J. T. Parsons, *Trends Cell Biol.* **3**, 258 (1993).
21. N. S. Bartfeld, E. B. Pasquale, J. E. Geltosky, L. R. Languino, *J. Biol. Chem.* **268**, 17270 (1993). IRS-1 and the 190-kD protein have different electrophoretic mobilities, and the 190-kD protein is not recognized by any of the three antibodies to IRS-1 we tested (K Vuori and E. Ruoslahti, unpublished data).
22. The mAbs LM609 (anti-$\alpha_v\beta_3$ complex) and P3G2 (anti-$\alpha_v\beta_5$ complex) have been described in E. A. Wayner, R. A. Orlando and D. A. Cheresh [*J. Cell Biol.* **113**, 919 (1991)]. All other integrin mAbs were from Telios Pharmaceuticals (San Diego, CA).
23. HIRcB cells were grown to 80% confluency in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 500 nM methotrexate, incubated in DMEM + 0.1% bovine serum albumin (BSA) for 36 hours, and either left quiescent or stimulated with 100 nM insulin (Sigma) for 3 min. Cells were rinsed with phosphate-buffered saline (PBS), frozen in liquid nitrogen, and lysed in 20 mM tris-HCl (pH 8.0), 1% NP-40, 10% glycerol, 137 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, aprotinin (0.1 U/ml), leupeptin (10 μg/ml), and pepstatin A (4 μg/ml). In all figures, anti-$\alpha_v\beta_3$ refers to anti-$\alpha_v\beta_3$ 237, and anti-$\alpha_v\beta_3$(2) refers to anti-$\alpha_v\beta_3$ 1343.
24. Anti-$\alpha_v\beta_3$ 237 immunocomplexes from insulin-treated cells were resuspended in 10 mM tris-HCl (pH 7.5), 2% SDS, 1 mM sodium orthovanadate, and 50 mM sodium fluoride and heated at 95°C for 5 min. The supernatant was diluted 10-fold in 10 mM tris-HCl (pH 7.5) and reprecipitated with anti-$\alpha_v\beta_3$ 237. Similar results were obtained with anti-$\alpha_v\beta_3$ 1343 and anti-$\alpha_{IIb}\beta_3$ [E. Dejana *et al.*, *J. Cell Biol.* **107**, 1215 (1988)].
25. Anti-pTyr py20, Grb2 mAb, and polyclonal antibody against the 85-kD subunit of PI-3 kinase were from Transduction Laboratories (Lexington, KY). The ^{125}I-labeled anti–mouse IgG was from Amersham.
26. After insulin stimulation, HIRcB cells (5×10^8) were lysed in 100 mM octyl-β-D-glucopyranoside in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and the phosphatase and protease inhibitors in (*23*). Chromatography was performed as in R. Pytela, M. D. Pierschbacher, S. Argraves, S. Suzuki, and E. Ruoslahti [*Methods Enzymol.* **144**, 475 (1987)].
27. Plates were coated with vitronectin (10 μg/ml) or type I collagen for 12 hours and then treated with 0.5% BSA for 2 hours. Adhesion to vitronectin was inhibited by anti-$\alpha_v\beta_3$ but not anti-$\alpha_5\beta_1$, and adhesion to collagen was inhibited by anti-β_1. Cells were greater than or equal to 85% viable on both vitronectin and collagen after 36 hours of incubation.

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Exocytosis in Spermatozoa in Response to Progesterone and Zona Pellucida

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Exocytosis in mammalian spermatozoa (the acrosome reaction) is a process essential for fertilization. Both progesterone and zona pellucida induce exocytosis in spermatozoa, which may encounter both during penetration of the oocyte's vestments. When mouse spermatozoa were exposed first to progesterone and then to zona pellucida, exocytosis was enhanced to a greater degree than that seen when the agonists were presented together or in the inverse order, which suggests that the steroid exerts a priming effect. Progesterone similarly primed the generation of intracellular messengers evoked by zona pellucida. The effects triggered by progesterone were mimicked by γ-aminobutyric acid (GABA) and were blocked by bicuculline, which indicates that the steroid acts on a $GABA_A$ receptor.

At fertilization, the spermatozoon undergoes exocytosis in response to an oocyte-derived signal or signals (*1*). However, two conflicting views exist regarding the agonist responsible for triggering exocytosis. The generally accepted view is that, after traversing the cumulus oophorus, the spermatozoon is stimulated by the zona pellucida (ZP) (*1*), and exposure to ZP leads to tyrosine phosphorylation of p95 (*2*) and activation of a guanosine triphosphate (GTP)–binding protein (G_i class) (*3*). However, targets for these transducing mechanisms have not been identified. An alternative view states that progesterone, trapped in or produced by the cumulus oophorus, can also initiate exocytosis (*4*). Progesterone action is specific (*5–7*), is mediated by a surface receptor (*8*), and also leads to phosphorylation of p95 (*9*); G protein activation, however, does not take place (*10*). We tested whether these agonists interact at an early stage during exocytosis and examined which messengers are elicited by progesterone or ZP and whether these agonists activate different pathways for the generation of such messengers.

Mouse spermatozoa undergo exocytosis of the acrosome when exposed to ZP (Fig. 1) (*1*). Mouse spermatozoa capacitated in vitro (*11*) also undergo exocytosis when exposed to progesterone (Fig. 1) (*12*). To investigate how the actions of these ago-

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