Integrin Heterodimer and Receptor Complexity in Avian and Mammalian Cells

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Abstract. We report data showing that the integrin receptor complex in chickens contains several discrete heterodimers all sharing the β1-integrin subunit combined separately with different α-subunits. Using antisera to synthetic peptides based on cDNA sequences of chicken and human α-integrin subunits to analyze the integrin complement of avian and mammalian cells, we show that band 2 of the chicken integrin complex contains α-subunits related to both α2- and α5-subunits of human integrins. α2β1 and α5β1 have both previously been shown in human cells to be fibronectin receptors and α5β1 can also act as a receptor for laminin and collagen. We also provide evidence for the presence, in band 1 of the chicken integrin complex, of a third integrin α-subunit which is also α5 related. This integrin subunit exists in a separate heterodimer complex with β1 and binds to fibronectin-affinity columns. These results provide explanations for published data showing that the avian integrin complex contains receptor activity for a variety of extracellular matrix proteins. We conclude that the chicken integrin complex comprises a set of β1-integrin heterodimers equivalent to the human VLA antigens and includes at least two fibronectin receptors. Finally, we show that chicken embryo fibroblasts also contain a β1-class integrin related to the RGD receptors defined in various human cells.

The family of cell surface receptors known as integrins includes multiple receptors for extracellular matrix proteins and the interactions of integrins with these extracellular ligands play important roles in cellular adhesion (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Buck and Horwitz, 1987). Integrins are transmembrane proteins composed of two subunit types, α and β. They fall into at least three major subclasses, each characterized by a common β-subunit and a set of α-subunits.

The ligand specificities of some αβ-complexes have been defined but others remain unclear. In particular, a complex of proteins was isolated several years ago from chicken cells using antibodies which block adhesion to extracellular matrix proteins (Neff et al., 1982; Greve and Gottlieb, 1982; Knudsen et al., 1985). This chicken integrin complex contains proteins which interact with fibronectin, collagen, and laminin (Horwitz et al., 1985; Akiyama et al., 1986; Buck and Horwitz, 1987), but it remains unclear whether there is a single complex with multiple affinities or whether there is a mixture of several different heterodimers each with distinct ligand specificity.

The β-subunit of the chicken integrin complex has been cloned (Tamkun et al., 1986) and is homologous in sequence with the β-subunit of an integrin receptor from human cells, isolated by its affinity for fibronectin (Pytela et al., 1985a; Argraves et al., 1987). Comparison of these sequences with the sequences of other integrin β-subunits (Fitzgerald et al., 1987; Kishomoto et al., 1987; Law et al., 1987; DeSimone and Hynes, 1988) has allowed classification based on three β-subunits (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). We designate the β-subunit of the chicken integrin complex β1; as mentioned, it is clearly the avian equivalent of the β-subunit of the so-called human "fibronectin receptor." Immunological evidence indicates that these two β1-subunits are equivalent to the β-subunit of a set of human antigens known as VLA antigens which have at least six distinct α-chains (α1–α6) (Hemler et al., 1987, 1988; Takada et al., 1987, 1988).

These data lead to a picture in which the integrin β1-subclass comprises at least six separate αβ-complexes. Some of these complexes have been assigned ligand specificities in human cells. The so-called human fibronectin receptor appears to be α5β1 (Pytela et al., 1985a; Takada et al., 1987; Argraves et al., 1987; Wayner et al., 1988). However, there is at least one other fibronectin receptor in the same integrin subclass, namely α2β1. This heterodimer mediates adhesion to fibronectin, laminin, or collagen (Wayner and Carter, 1987; Wayner et al., 1988; Takada et al., 1988). Very recently, laminin receptors from the β1-class of integrins have been isolated (Gehlsen et al., 1988; Ignatius and Reichardt, 1988). Finally, α3β1 appears to be a collagen receptor (Nieuwenhuis et al., 1985; Kunicki et al., 1988). Other β1-integrins are not well defined as to their ligand specificity and it is unclear how many of the human α-subunits are related...
Materials and Methods

Cells

Chicken embryo fibroblasts (CEFs) were prepared from day 11 embryos (Spafas, Inc., Norwich, CT) as described (Rein and Rubin, 1968). Cells were grown in DME plus 5% FCS (Gibco Laboratories, Grand Island, NY) and used between passages 3 and 9. MG63 human osteosarcoma cells (Bilioua et al., 1977) were grown in DME plus 10% FCS.

Purification of Chicken Integrin Complex

Chicken integrin complexes were purified from chicken embryos following the procedure of Knudsen et al. (1985) with minor modifications. Chicken embryos from day 10 to 14 were decapitated, eviscerated, and their limbs removed while on ice. The tissue was weighed, and then homogenized with 10 ml per 30 embryos of PBS in a Waring blender (Waring Products; Dynamics Corp., New Hartford, CT) at 4°C twice for 15 s. Solubilization buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM CaCl₂, 2 mM PMSF, 0.03 U/ml aprotinin, and either 0.5% NP-40 or 50 mM β-octylglucoside [BOG; Calbiochem-Behring Corp., La Jolla, CA]) was added in the amount of 3 ml/g original wet weight of tissue. The homogenate was filtered through three layers of cheesecloth, stirred for 1 h at 4°C, then spun at 3000 g for 10 min and the supernatant further spun at 60,000 g for 45 min. White fluffy material was aspirated off the top of the supernatant and the latter was passed through Whatman Inc. (Clifton, NJ) 3 ml filter paper, acidified to 20 mM acetic acid (pH 5.5), and left at 4°C for 45 min. Insoluble material was removed by centrifugation at 12,000 g for 10 min and the supernatant neutralized to pH 8.0 with 1 M Tris-HCl. The resulting extract was clarified when necessary by centrifugation (60,000 g for 30 min) and/or by passage through a 0.45-μm Nalgene filter. The extract was either used immediately or stored frozen at −35°C.

CSAT monoclonal antibody affinity columns were prepared by coupling of 10 mg/ml isolated IgG from CSAT hybridomas (Neff et al., 1982) to CNBr-activated Sepharose 4B. Chicken embryo extract was passed over a 1- or 2-ml column at a flow rate of 5–10 ml/h at 4°C. The column was then washed with 50 ml of wash buffer (10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.5 mM CaCl₂, and either 0.5% NP-40 or 50 mM BOG). The column was eluted with 50 mM diethylamine buffer, pH 11.5, with 0.15 M NaCl, 0.5 mM CaCl₂, and either 0.5% NP-40 or 50 mM BOG. Fractions were immediately neutralized to pH 8.0 with 1 M Tris, pH 6.8, and analyzed on SDS-PAGE gels. The peak fractions were pooled and frozen at −35°C.

Peptide Synthesis

Synthetic peptide corresponding to the COOH-terminal 14 residues of chicken band 2 (α,2) was kindly provided by C. Buck (Wistar Institute, Philadelphia, PA). The α,2-23mer corresponding to the human α,2 COOH terminus was synthesized by Peninsula Laboratories, Inc. (Belmont, CA). GRGESP, GRGDSP, RNGESQ (a peptide analogous to GRGDSP but from the next type III repeat, II–1; Schwarzbauer et al., 1983; Kornblihtt et al., 1985), and a peptide corresponding to the COOH-terminal 13 amino acids of the human α,2-sequence were synthesized using a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) using solid-phase t-boc chemistry. Peptides were deprotected and cleaved from the solid support by treatment with anhydrous TFA and phenol with shaking. They were then purified further by reverse-phase HPLC chromatography on a Vydac C18 column (Rainin Instruments Co., Woburn, MA), eluted with a 0–60% acetonitrile gradient in 0.1% trifluoroacetic acid.

Production of Antipeptide Sera

The α,2-14mer peptide was coupled to keyhole limpet hemocyanin (KLH; Calbiochem-Behring Corp.) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Sigma Chemical Co., St. Louis, MO) essentially as described (Staraos et al., 1986). 2 mg of KLH and 15 mg of peptide were mixed in 4 ml of PBS. After adjusting the pH to 5.5 with HCl, EDC was added to a final concentration of 0.1 M. The reaction was stirred overnight at room temperature and quenched with 0.5 M ethanolamine for 1 h. After exhaustive dialysis against 0.05 M NH₄HCO₃, pH 7.5, the mixture was lyophilized and reconstituted in 1 ml of water. α,2-Cys-23mer was conjugated to KLH with m-maleimidobenzoyl-1-succinimide ester (Pierce Chemical Co., Rockford, IL) using a procedure described previously (Marcantonio and Hynes, 1988).

For injection, 200 μg of peptide conjugate in PBS were mixed with 1 ml of complete Freund’s adjuvant (Gibco Laboratories) by sonication and injected subcutaneously at multiple sites along the dorsal midline of rabbits. At 3–4 wk intervals, booster injections of 100 μg of protein in incomplete adjuvant were given. Significant antibody titers were observed after two boosts for the α,2-23mer, and after five boosts for the α,2-14mer.

Other Antibodies

Rabbit anti-α2 cytoplasmic domain sera were prepared as described (Marcantonio and Hynes, 1988). Rabbit anti-α5 COOH terminus (13mer) serum was donated by W. S. Argalves and E. Ruoslabi (La Jolla Cancer Research Foundation, La Jolla, CA). Monoclonal anti-extracellular matrix receptor (ECMR) 1 (P1B5) (Wayner and Carter, 1987) was gift of W. G. Carter and E. Wayner (Fred Hutchinson Cancer Research Center, Seattle, WA) and CSAT antibody (Neff et al., 1982) was a gift of C. Buck (Wistar Institute) and R. Horvitz (University of Illinois, Urbana, IL). Monoclonal anti-RGD receptor (LM609; Cheresh and Spiro, 1987) was a gift of D. Cheresh (Research Institute of Scripps Clinic, La Jolla, CA).

Iodination and Immunoprecipitation

Cells were labeled with 125I and lactoperoxidase (Sigma Chemical Co.) in monolayers as described (Hynes, 1973). 107 cells and 1.0 mCi/ml were used per experiment. Cells were washed three times with PBS with 1 mM CaCl₂ and 1 mM MgCl₂, resuspended in 1 ml of extraction buffer (50 mM Tris, pH 8.0, 0.15 mM NaCl, 0.5 mM CaCl₂, 0.5% NP-40), incubated for 15 min on ice, and then sedimented for 10 min at 10,000 g. The supernatant was preincubated with 50 μl of protein A-Sepharose for 5 min and the beads sedimented for 2 min at 10,000 g. The resulting supernatant was used for immunoprecipitation. Typically, 5 × 10⁶ TCA-precipitable counts were used per sample.

Integrin complex purified from day 11 chicken embryos was labeled with 125I using iododecades (Pierce Chemical Co.) and desalted using a 10 × 0.5-cm G-50 column (Pharmacia Fine Chemicals, Piscataway, NJ) with extraction buffer. The peak fraction containing the void volume was pooled and used directly for immunoprecipitation.

Immunoprecipitation of native and denatured labeled cell extracts or of purified integrin complex was performed as described previously (Marcantonio and Hynes, 1988).

Western Immunoblotting

Samples of chicken embryo extract or purified integrin were solubilized in electrophoresis sample buffer and run on 7% nonreducing SDS-PAGE gels with 4% stacking gels (Laemmli, 1970). Proteins were electrophoretically transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) overnight at 500 mA (Towbin et al., 1979). Filters were incubated with rocking for 1-3 h. For peptide competition experiments, synthetic peptides were added at 100 μg/ml. Each filter was washed three times with 15 min in blocking buffer, introduced into miniblotter channels, and filters were incubated with rocking for 1-3 h. For peptide competition experiments, synthetic peptides were added at 100 μg/ml. Each filter was washed three times with 15 min in blocking buffer, introduced into miniblotter channels, and filters were incubated with rocking for 1-3 h. For peptide competition experiments, synthetic peptides were added at 100 μg/ml. Each filter was washed three times with 15 min in blocking buffer, introduced into miniblotter channels, and filters were incubated with rocking for 1-3 h. For peptide competition experiments, synthetic peptides were added at 100 μg/ml. Each filter was washed three times with 15 min in blocking buffer, introduced into miniblotter channels, and filters were incubated with rocking for 1-3 h. For peptide competition experiments, synthetic peptides were added at 100 μg/ml. Each filter was washed three times with 15 min in blocking buffer, introduced into miniblotter channels, and filters were incubated with rocking for 1-3 h.
**Results**

**cDNA Clones and Sequence of an Avian Integrin \( \alpha \)-Subunit**

In our original isolation of chicken integrin cDNA clones using a polyclonal antibody (Tamkun et al., 1986) we reported three clones, two of which were shown to encode the \( \beta \)-subunit. The third clone, I, was used to isolate related cDNA clones and several of these were sequenced. Fig. 1 shows a partial amino acid sequence deduced from the sequence of these clones. The sequence comprises the COOH-terminal 179 residues of an integrin \( \alpha \)-subunit and corresponds with the light chain of an \( \alpha \)-subunit, as can be seen by comparison with the sequences of several other \( \alpha \)-subunits (Fig. 1). The sequence, while clearly homologous with \( \alpha \)-subunits in general, is not closely related to the sequence of any specific one of the human \( \alpha \)-subunits which have been published. In particular, the sequence is distinct from that of the human fibronectin receptor \( \alpha \)-subunit (\( \alpha_\alpha \)), the only published sequence for an \( \alpha \)-subunit of the \( \beta \) sub-class (Fig. 1). Data to be presented below lead us to conclude that the chicken \( \alpha \)-sequence corresponds with the human \( \alpha \_\alpha \)-subunit and, for clarity, we will refer to this subunit as \( \alpha_\alpha \) (see Discussion).

**\( \alpha \)-Subunit-specific Antisera**

To identify the \( \alpha \)-subunit encoded by this cDNA, we obtained a synthetic peptide corresponding with the COOH-terminal 14 residues (Fig. 1). We also obtained a synthetic peptide corresponding with the COOH-terminal 23 residues of the human \( \alpha \_\gamma \)-sequence (Fig. 1). Polyclonal antisera were raised against each of these peptides conjugated to KLH and tested by Western blotting and immunoprecipitation.

Fig. 2A shows that antisera to the chicken cDNA \( \alpha \_\gamma \)-sequence recognizes predominantly band 2 of the chicken integrin complex on Western blots. Fig. 3 (lane f) shows immunoprecipitation by this antisera under denaturing conditions: it precipitates a band comigrating with band 2. Under non-denaturing conditions, the antisera precipitates material comigrating with both band 2 (\( \alpha_\alpha \)) and band 3 (\( \beta_\alpha \)) indicating the presence of an \( \alpha_\alpha/\beta_\alpha \)-heterodimer (Fig. 3, lane b).

On Western blots this antisera variably stains higher molecular weight bands which we suspect arise artefactually during isolation and concentration of purified integrin (Fig. 2), since they are not observed in blots of whole embryo homogenates (data not shown). The presence of this \( \alpha_\alpha \)-subunit in band 2 of the chicken integrin complex agrees with immunological data showing cross-reaction of anti-human \( \alpha \_\gamma \)-antibodies with band 2 (Takada et al., 1987).

Antiserum raised against human \( \alpha_\alpha \)-23mer peptide cross-reacted with both bands 1 and 2 of the purified chicken integrin complex on Western blots (Fig. 2B) or by immunoprecipitation (Fig. 3). Two different antisera gave similar results, although one of them showed a stronger bias toward band 1 than the other in immunoprecipitation assays (Fig. 3, lanes d and e). This cross-reactivity with two different bands was somewhat surprising. Accordingly, we obtained a third antiserum raised against a shorter \( \alpha_\alpha \)-peptide (a generous gift of S. Argraves and E. Ruoslahti, La Jolla Cancer Research Foundation). This antiserum was raised against the COOH-terminal 13 residues of the human \( \alpha \_\gamma \)-sequence (see Fig. 1). As shown in Fig. 2A and B, this antiserum recognized a single \( \alpha \)-subunit band on Western blots. This \( \alpha \_\gamma \)-cross-reactive subunit (\( \alpha_\alpha \)) comigrated with the lower band recognized by the two antisera against the 23 residue \( \alpha_\gamma \)-peptide and was slightly larger than the band recognized by the putative \( \alpha \)-antisemur. In immunoprecipitation experiments, this antiserum precipitated two bands comigrating with bands 2 and 3 of the integrin complex (Fig. 3, lane c). This indicates the existence of a distinct \( \alpha_\alpha/\beta_\alpha \)-dimer recognized by this antiserum.

These results lead to several conclusions. First, at least...
Figure 1. Comparison of chick integrin α-subunit with several human α-subunits. The COOH-terminal 179 amino acid residues of a chicken integrin α-subunit cDNA clone (chick α3) are compared with sequences of three human α-subunits. Fibronectin receptor (FNR, α3; Argraves et al., 1987; Fitzgerald et al., 1987), vitronectin receptor (FNR, αv; Suzuki et al., 1987), and platelet glycoprotein IIb (IIb; Poncz et al., 1987; Fitzgerald et al., 1987). Several homologies are highlighted; including a set of three cysteine residues (*), the transmembrane domain (heavy lines), and the dibasic residues which denote the start of the cytoplasmic domain (+). The known or suggested cleavage sites of α3, αv, and platelet glycoprotein IIb are marked (arrowsheads). These cleavages generate the COOH-terminal light chain of these α-subunits. The sequence of α3 also suggests a cleavage site after a dibasic sequence. The peptides used as immunogens are underlined: α3-14mer, α3-23mer, and α3-13mer.

two different β-subunits are present in the band 2 region of the gels. One (αβ3) is recognized by three different antisera against the human αβ-sequences and a second, slightly smaller one is recognized by antisera against the chicken αβ-peptide. Second, antisera against a longer human αβ-peptide recognize, in addition, a third α-subunit which migrates in the band 1 region (αβ3). Since the sera were tested in parallel on the same preparations of integrins, the reaction with band 1 of our two antisera raised against the αβ23mer cannot be due to aggregation or anomalous migration of the smaller αβ cross-reactive species so that it migrates in the band 1 region. Any such aggregates should also be recognized by Argraves’ antisera to the αβ-13mer which does not cross-react with band 1. This suggests, rather, that some epitope in the first 10 residues of the 23mer peptide is present in a different, larger αβ-subunit in the band 1 region.

To test the epitope specificity of the antisera, we performed peptide competition experiments. Fig. 2 C, antisera 1, shows that the anti-αβ3-serum is blocked by the αβ3-peptide but not by either of the αβ-peptides. The different anti-αβ-
Figure 3. Immunoprecipitation of integrins purified from chicken embryo extract. Integrin complexes were purified from chicken embryo extract using a CSAT-Sepharose column. Purified integrins were \( ^{125}\text{I}-\)labeled and incubated with (lane a) anti-\(\beta_1\)-peptide serum; (lane b) anti-\(\alpha_2\)-peptide serum; (lane c) anti-\(\alpha_3\)-13mer peptide serum; (lane d) anti-\(\alpha_2\)-13mer peptide serum #160; (lane e) anti-\(\alpha_3\)-23mer peptide serum #161; (lane f) anti-\(\alpha_3\)-23mer peptide serum after SDS denaturation of integrins; or (lane g) anti-\(\alpha_2\)-23mer antiserum #160 after SDS denaturation of integrins. The samples were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE (nonreduced) followed by autoradiography of the dried gels. Specific sets of \(\alpha\beta\)-heterodimers are precipitated under non-denaturing conditions with the \(\alpha\)-antisera, while only \(\alpha\)-subunits are recovered under denaturing conditions. Molecular mass markers indicated by dots at left are from top to bottom: myosin (200 kD), \(\beta\)-galactosidase (116 kD), phosphorylase B (93 kD), and BSA (68 kD).

Groups working on mammalian cells, we also tested the anti-peptide sera on extracts of surface-labeled MG63 cells (Fig. 4).

All three anti-\(\alpha_3\)-peptide sera reacted with a broad fuzzy band and immunoprecipitated the \(\beta_1\)-subunit in a complex with this \(\alpha_3\)-subunit (Figs. 4, A, lane f, and B). The \(\alpha_3\)-band frequently appeared as a closely spaced doublet with all three anti-\(\alpha_3\)-sera but we did not observe differential reactivities among the three anti-\(\alpha_3\)-sera when tested on human cells (Fig. 4 B). All three were blocked by either the \(\alpha_3\)-23mer peptide or the \(\alpha_3\)-13mer peptide suggesting that the major epitopes recognized by these three antisera in human cells lie in the COOH-terminal 13 residues. There was no evidence for the presence in MG63 cells of larger \(\alpha_3\)-related subunits reactive only with antisera to the large peptide.

The anti-\(\alpha_3\)-peptide serum precipitated a sharp \(\alpha\)-band which was also complexed with the \(\beta_1\)-subunit (Fig. 4 A, lane b). The major \(\alpha\)-band in MG63 cells was not \(\alpha_3\)-related but reacted with the anti-\(\alpha_3\)-serum (compare Figs. 4 A, lane a, and B). These results are consistent with previous findings (Hemler et al., 1987; Takada et al., 1987) showing that \(\alpha_3\) is a major subunit on these cells while \(\alpha_3\) is present in relatively lower quantities. The human \(\alpha_3\)-subunit is also recognized by a monoclonal antibody, PIBS (Wayner and Carter, 1987), as shown in Fig. 4 A (lane d) and preclearing of the extracts with PIBS abolished subsequent precipitation with the antiserum to the \(\alpha_3\)-peptide (Fig. 4 A, lane c) but not with the anti-\(\alpha_3\)-peptide (Fig. 4 A, lane f). These results confirm the identity of the chicken \(\alpha\)-subunit as \(\alpha_3\).

Formation of Discrete \(\alpha\beta\)-Complexes during Biosynthesis

Data presented in the preceding sections provide evidence that the chicken integrin complex contains several different \(\alpha\beta\)-heterodimers and argue strongly that the complex is not a heterotrimer or some higher oligomer containing all three of bands 1–3. To investigate this question further, we examined the formation of \(\alpha\beta\)-heterodimers during biosynthesis. CEFs were incubated in \(\text{[35S]}\)methionine for 30 min and then chased in unlabeled methionine for varying periods before extraction and immunoprecipitation with antiserum against \(\beta_1\) or \(\alpha_3\). The results are shown in Fig. 5. After the pulse, one can detect precursor to \(\alpha_3\), using the anti-\(\alpha_3\)-14mer serum (Fig. 5 C, lane 0). At the same time the anti-\(\beta_1\)-serum precipitates precursor \(\beta_1\), and what appear to be at least two different precursors to \(\alpha\)-subunits (Fig. 5 B, lane 0). After 1 h of chase, antiserum to \(\beta_1\) precipitates precursor \(\beta_1\), a small amount of mature \(\beta_1\), and mature \(\alpha_3\)'s of both band 1– and band 2–size classes. The anti-\(\alpha_3\)-serum precipitates only the mature \(\alpha_3\)-subunit (in the band 2 region) along with some precursor \(\beta_1\) and some mature \(\beta_1\). Rather slowly over the next few hours, the precursor \(\beta_1\) matures to its final form which is precipitated by both antiserum (\(\beta_1\) and \(\alpha_3\)). Comparison of Fig. 5, B and C, shows clearly that the antibody against \(\alpha_3\)-peptide selectively precipitates an \(\alpha_3\beta_1\)-complex at all chase times while the \(\beta_1\)-antibody precipitates a mixture of several different \(\alpha\)-subunits along with the \(\beta_1\)-subunit. This provides further evidence in support of independent, discrete \(\alpha\beta\)-heterodimers in chicken cells. The complex pattern of bands precipitated by antibodies reactive with \(\beta_1\) (including CSAT and JG22 monoclonals; Buck et

**Figure 3.** Immunoprecipitation of integrins purified from chicken embryo extract. Integrin complexes were purified from chicken embryo extract using a CSAT-Sepharose column. Purified integrins were \( ^{125}\text{I}-\)labeled and incubated with (lane a) anti-\(\beta_1\)-peptide serum; (lane b) anti-\(\alpha_2\)-peptide serum; (lane c) anti-\(\alpha_3\)-13mer peptide serum; (lane d) anti-\(\alpha_2\)-13mer peptide serum #160; (lane e) anti-\(\alpha_3\)-23mer peptide serum #161; (lane f) anti-\(\alpha_3\)-23mer peptide serum after SDS denaturation of integrins; or (lane g) anti-\(\alpha_2\)-23mer antiserum #160 after SDS denaturation of integrins. The samples were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE (nonreduced) followed by autoradiography of the dried gels. Specific sets of \(\alpha\beta\)-heterodimers are precipitated under non-denaturing conditions with the \(\alpha\)-antisera, while only \(\alpha\)-subunits are recovered under denaturing conditions. Molecular mass markers indicated by dots at left are from top to bottom: myosin (200 kD), \(\beta\)-galactosidase (116 kD), phosphorylase B (93 kD), and BSA (68 kD).

**Mammalian \(\alpha\)-Subunits**

To relate these results on chicken integrins to those of other...
Figure 4. Immunoprecipitation of integrin complexes from MG63 cells. (A) Identification of \( \alpha_3\beta_1 \)-complex. Extracts of \(^{125}\)I surface-labeled MG63 cells were incubated with (lane a) anti-\( \beta_1 \)-peptide serum with 10 \( \mu \)g of \( \alpha_5 \)-peptide added; (lane b) anti-\( \alpha_5 \)-peptide serum; (lane c) anti-\( \alpha_5 \)-peptide serum with 10 \( \mu \)g of \( \alpha_5 \)-peptide; (lane d) anti-ECMR I antibody, PIB5, plus rabbit anti-mouse IgG-Sepharose; (lane e) anti-\( \alpha_5 \)-peptide serum after two cycles of preclearing with anti-ECMR I antibody plus rabbit anti-mouse IgG-Sepharose; and (lane f) anti-\( \alpha_5 \)-23mer peptide serum after preclearing with anti-ECMR I antibody PIB5. The samples (a–c, e, and f) were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE (nonreduced) followed by autoradiography. Molecular mass markers shown by dots at the right as in Fig. 3. (B) Immunoprecipitation of \( \alpha_5\beta_1 \)-complexes. Extracts of \(^{125}\)I surface-labeled MG63 cells were incubated with (lane a) anti-\( \beta_1 \)-peptide serum with 10 \( \mu \)g each (+) of both \( \alpha_5 \)-13mer and \( \alpha_5 \)-23mer peptides added; (lane b) anti-\( \alpha_5 \)-23mer peptide serum; (lanes c and d) anti-\( \alpha_5 \)-23mer peptide serum #160; (lanes c and d) anti-\( \alpha_5 \)-23mer peptide serum #160 with 10 \( \mu \)g (+) of either \( \alpha_5 \)-13mer peptide (lane c) or \( \alpha_5 \)-23mer peptide (lane d); (lane e) anti-\( \alpha_5 \)-23mer peptide serum #161; (lanes f and g) anti-\( \alpha_5 \)-23mer peptide #161 serum with 10 \( \mu \)g (+) of either \( \alpha_5 \)-13mer peptide (lane f) or \( \alpha_5 \)-23mer peptide (lane g); (lane h) anti-\( \alpha_5 \)-13mer peptide serum; (lanes i and j) anti-\( \alpha_5 \)-13mer peptide serum with 10 \( \mu \)g (+) of either \( \alpha_5 \)-13mer peptide (lane i) or \( \alpha_5 \)-23mer peptide (lane j). The samples were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE (nonreduced) followed by autoradiography. Molecular mass markers are shown by dots at left.

The three \( \alpha_5 \)-antisera all react with \( \alpha_5\beta_1 \)-complexes and this precipitation is blocked by either \( \alpha_5 \)-13mer or \( \alpha_5 \)-23mer peptides.

al., 1986) is due to precipitation of a variety of different \( \alpha\beta \)-complexes.

The data in Fig. 5 also indicate that \( \alpha\beta \)-complexes form in the cells before complete processing of the subunits. Thus, antibodies against \( \beta_1 \), precipitate precursor \( \alpha \)-subunits at early times (Fig. 5 B, lane 0) and, conversely, antibody against \( \alpha_5 \) precipitates precursor \( \beta_1 \) (Fig. 5 C, lane 1). This is more clearly seen in a similar experiment (Fig. 6) performed on MG63 cells. These cells have a simpler pattern of \( \alpha \)-subunits among which \( \alpha_5 \) predominates (Fig. 4). At 0, 1, and 2 h of chase the precursor form of \( \alpha_5 \) is evident. The precursor form of \( \beta_1 \) is coprecipitated by antibodies to \( \alpha_5 \) (Fig. 5 C, lanes 1 and 2) although it is not labeled stoichiometrically with the \( \alpha \)-subunit. This is because mammalian cells contain a larger, intracellular pool of presynthesized \( \beta_1 \)-subunit which can also combine with \( \alpha \)-subunits but does not appear as a labeled species; it is seen by immunoblotting with anti-\( \beta_1 \)-sera (data not shown). The presence of this large pool of immature \( \beta_1 \)-subunits is reflected in the very slow processing during the chase of the precursor to \( \beta_1 \) (Fig. 6 B). Labeled precursor \( \beta_1 \) is still in excess over mature \( \beta_1 \) at 12 h of chase and still present after 24 h (Fig. 6). In contrast, the avian \( \beta_1 \)-subunit is largely in the mature form by 6 h of chase and completely matured by 12 h (Fig. 5). However, apart from these differences in kinetics, the process of \( \alpha\beta \)-assembly appears similar in avian and mammalian cells (see also Discussion).

**Ligand Specificity of Chicken Integrins**

Previous attempts to demonstrate direct interaction of purified chicken integrins with affinity columns of candidate ligands have been complicated by the low affinity of the interactions (Akiyama et al., 1985). To demonstrate these low affinity interactions, Horwitz and colleagues (Horwitz et al., 1985; Buck et al., 1986) have used equilibrium-gel filtration chromatography. We have attempted to fractionate purified, iodinated, chicken integrin complex by affinity chromatography and have similarly observed low levels of interaction. However, we have been able to demonstrate interaction of chicken integrins with affinity columns using either total extracts of surface-labeled CEFs, or purified integrins bound directly without prior iodination.

Fig. 7 A shows fractionation of an extract of surface-labeled CEFs on a column of GRGDSPC-Sepharose. Bound integrins were eluted by GRGDSPC but not by GRGESP, as shown for other cell types (Pytel et al., 1985b, 1986; 1987).
Cheresh, 1987; Cheresh and Spiro, 1987; Lawler et al., 1988). The bound material was analyzed by immunoprecipitation with various antibodies (Fig. 7 B). The major avian integrin complex selected by the GRGDSP column appears distinct from the chicken integrin complex defined by the CSAT monoclonal antibody (data not shown) or by antisera against the cytoplasmic domains of $\beta_1$ or $\alpha_3$ (Fig. 7 B). The avian RGD-binding complex is, however, precipitated by a monoclonal antibody (LM609) raised against a $\beta_3$-class integrin complex isolated from human melanoma cells (Cheresh and Spiro, 1987) as shown in Fig. 7 B.

Therefore, the GRGDSP column selects a $\beta_3$-integrin from extracts of chicken cells. This integrin is presumably the avian homologue of human receptors which bind to GRGDSP columns (Pytela et al., 1985b, 1986; Cheresh, 1987; Cheresh and Spiro, 1987; Lawler et al., 1988). Avian $\beta_1$-integrins are poorly bound by this column, although we frequently detected a trace amount of what appears to be $\beta_1$ in the GRGDSP eluates of this column (data not shown).

We turned next to affinity chromatography on columns coupled with the FNf (FNf-Sepharose). In early experiments using buffers containing MgCl$_2$ and CaCl$_2$, we observed only a small amount of binding of chicken integrins as reported by others (Akiyama et al., 1985). However, substitution of MnCl$_2$ as suggested by Gailit and Ruoslahti (1988) greatly enhanced the binding. Fig. 8, A and B, shows fractionation of BOG extracts of iodinated CEFs on columns of FNf-Sepharose. Much more material bound and was eluted specifically by GRGDSP when the buffers contained MnCl$_2$. The bound species migrated in the positions of bands 1 and 3. We repeated the experiment with integrins purified from chicken embryo extracts. While the starting sample contained all three of bands, 1, 2, and 3, and subunits reacting with each of the antisera to $\alpha_3$ and $\alpha_5$ (Fig. 9 A),

Figure 5. Biosynthesis and assembly of integrin complexes in CEF. CEFs were labeled with $[^{35}S]$methionine for 30 min and then chased for various times (h) with growth medium as indicated at the bottom of the figure. Labeled cells were then washed, extracted, and immunoprecipitated using anti-$\beta_1$-peptide serum (B) or anti-$\alpha_3$-peptide serum (C) followed by SDS-PAGE (nonreduced) and fluorography. Molecular mass markers (as in Fig. 3) are indicated by dots on the left and integrin bands 1, 2, and 3 are marked on the $^{125}$I surface-labeling profile (A).

Figure 6. Biosynthesis and assembly of integrin complexes in human MG63 cells. MG63 cells were labeled with $[^{35}S]$methionine for 30 min and chased for various times (h) with growth medium as indicated at the bottom of the figure. Labeled cells were then washed, extracted, and immunoprecipitated with either anti-$\beta_1$-peptide serum (B) or anti-$\alpha_3$-peptide serum (C) followed by SDS-PAGE (nonreduced) and fluorography. (A) The mature integrin, $^{125}$I-surface-labeled profile is shown.
Figure 7. Affinity chromatography on GRGDSPC-Sepharose. (A) CEFS were iodinated and extracted with BOG buffer. The extracts were passed through a column of GRGDSPC-Sepharose; the column was then washed and eluted sequentially with GRGESP and GRGDSP. The latter peptide selectively eluted bands of approximate molecular masses of 130, 80, and 75 D. Molecular mass markers are indicated by dots from top to bottom: myosin (200 kD), phosphorylase B (97 kD), and BSA (68 kD). (B) The total extract or the GRGDSP-eluted material was immunoprecipitated with the antibodies indicated. The bound material was precipitated by LM609, a monoclonal antibody against β3-integrins. The lowest band was variable in different experiments and probably represents a degradation product.

the specifically bound and eluted material contained predominantly bands 1 and 3 (Fig. 9 B), which could be immunoprecipitated by antibodies to β1 or to human α2-23mer (Fig. 9 C). No material reactive with antisera to α3 or to α3-13mer was detected in the bound fractions (Fig. 9 C), although cross-reactive species were detected in the starting sample (Fig. 9 A) and in the unbound material (data not shown). When a similar experiment was performed with MG63 human cells, α3β1, which was reactive with all three of the anti-α-piptide sera, bound to the column, while α3β1 did not (Fig. 10).

Thus, both human and chicken α3β1-integrins fail to bind to FNf-Sepharose at physiological salt concentrations. α3-related integrins from both humans and chickens do bind to FNf-Sepharose but we have been unable to detect binding of the smaller α3 cross-reactive species (α3a) from chicken integrins. Whether this reflects the low levels present or another technical problem, or whether this integrin does not interact with FNf is currently unclear (see Discussion). However, it is clear that the larger α3-related β1-integrin from chickens (α3b) does bind to FNf-Sepharose and is, therefore, a fibronectin receptor by this criterion.

Discussion

We have presented data that establish the following points. (a) The chicken integrin complex originally defined by adhesion-blocking antibodies contains multiple different α-subunits each of which occurs as a heterodimer with the common β1-subunit. (b) The α-band originally described as band 2 contains at least two different α-species: a minor α2-reactive subunit; and another, slightly smaller, α-subunit which reacts with antibodies against a peptide defined by a chicken cDNA clone. We designate these two “band 2 α-subunits” αS and αS (see below). (c) Band 1 contains another α-subunit (αS) which is cross-reactive with some but not all antisera raised against human αS-sequences. This α-subunit complexed with β1 binds to columns of fibronectin cell-binding fragments. The αS-species appears to be distinct from αS. (d) CEFS also bear yet another integrin, from the β3-class, which binds to GRGDSP columns and is immunologically cross-reactive with human β3-integrin receptors isolated in similar fashion. (e) In both avian and mammalian cells, discrete αβ-complexes form before complete processing of the carbohydrates of the subunits, that is, in a pre-Golgi compartment.

Figure 8. Fibronectin affinity chromatography of CEF extracts. Cells were iodinated, extracted with BOG in the presence of MgCl2 and CaCl2 (A) or MnCl2 (B). Extracts were passed over columns of FNf-Sepharose. The columns were washed well and eluted with GRGDSP. Two bands were bound and eluted under both salt conditions, but much more material was recovered using buffers containing MnCl2. Migration positions of bands 1, 2, and 3 are marked.
Figure 9. Fibronectin affinity chromatography of purified chicken embryo integrins. Purified integrins from chicken embryo extracts were chromatographed on a column of FNf-Sepharose. After washing, the column was eluted with RNGESQ followed by GRGDSP. Starting material and eluted fractions were iodinated with Iodobeads and some samples were immunoprecipitated. (A). Starting material immunoprecipitated with the following antisera: lane a, anti-β1; lane b, anti-α3; lane c, anti-α5-13mer; lane d, anti-α5-23mer #160; and lane e, anti-α5-23mer #161. Migration positions of bands 1-3 and specific α- and β-integrin subunits are marked. (B) Elution profile. The total samples are shown without immunoprecipitation. Migration positions of bands 1-3 are marked. (C) Immunoprecipitation of peak eluted fractions (see B) with the same antisera as in A. The eluted bands comprise an α5β1-heterodimer. α3 and α5 are not detectable.

Integrin Heterodimer Complexity

One message derived from our data is that the integrins on avian cells are very similar to those on mammalian cells. Both β1- and β3-class integrins are present and the previously defined chicken integrin complex is the avian equivalent of the human VLA antigens. Both the VLA antigens and the chicken integrin complex have a common βr-subunit; the βr-subunits of the two species are 85% identical in sequence (Tamkun et al., 1986; Argraves et al., 1987; DeSimone and Hynes, 1988). The βr-subclass of integrins in both species is complex; at least six α-subunits have been defined on human cells and we present direct evidence here for three different α-subunits in chickens each of which forms a separate heterodimer with the βr-subunit (Fig. 3).

Figure 10. Fibronectin-binding integrins from MG63 cells. Material isolated from extracts of iodinated MG63 cells by affinity chromatography on a column of FNf was immunoprecipitated with the following antisera: lane a, anti-β1; lane b, anti-α3; lane c, anti-α5-13mer; lane d, anti-α5-23mer #160; and lane e, anti-α5-23mer #161. Note the presence of an αβ1-heterodimer reactive with all antisera against αr-peptides and the absence of α5β1.

How do the avian α-subunits relate to the better defined human VLA α-subunits? The chicken α-subunit cDNA clone (Fig. 1) defines an α-subunit which we have referred to as α3. The evidence is as follows. (a) Antibodies to a peptide defined by this sequence react with avian integrin band 2 which is known also to cross-react with antibodies to human VLA-α3 (Takada et al., 1987). (b) Antibodies to the chicken peptide cross-react with the major α-subunit on MG63 cells (Fig. 4 A); α3 is the major VLA-α-subunit on these cells (Hemler et al., 1987; Takada et al., 1987). (c) A monoclonal antibody, P1B5, which reacts with an extracellular matrix receptor (ECMR I) with affinity for fibronectin, laminin, and collagen (Wayner and Carter, 1987) has been shown to react with VLA-α3 (Takada et al., 1988) and preclears material reactive with our anti-chicken α3-peptide serum (Fig. 4 A). (d) Comparison of the chicken sequence with a partial sequence of a human cDNA clone isolated with antibodies to VLA-α3 (Hemler, M. E., and Y. Takada, personal communication) shows strong homology; 24 out of 30 identical residues in the COOH-terminal cytoplasmic domain sequence. These two clones also cross-react on Southern blots (Stepp, M. A., Y. Takada, M. E. Hemler, and R. O. Hynes, unpublished data) whereas α3 and α5 cDNA clones do not cross-react with each other. These data provide a reasonably strong argument that this is indeed the chicken α3-subunit, which is one constituent of band 2.

A slightly larger α-subunit (α5b) is recognized by three antisera raised against two different peptides from human α5 (Figs. 1-4). A complication arises from the fact that two of the antisera raised against human α5-peptides recognize, in addition, an even larger α-subunit in chickens (Figs. 2 and 3). This subunit (α5b) migrates in the band 1 region and is not recognized by Argraves' antisera raised against a shorter α5-peptide. Chicken α5b shares epitopes with human α5 which are contained in the last 12 residues of the cytoplasmic domain (EKAQLKPPATSDA; Fig. 1). In contrast, chicken α5b shares epitopes with human α5 which are contained in the first 10 residues of the cytoplasmic domain (KRSLPYGTAM; Fig. 1) but does not share epitopes con-
tained in the sequence EKAQLKPPATSDA (Fig. 2 C). The simplest interpretation of these results is that \( \alpha_a \) and \( \alpha_b \) in chickens represent two \( \alpha \)-subunits with different amino acid sequences, each of which happens to be related to the sequence of human \( \alpha_5 \).

Alternative explanations are that some form of posttranslational modification or experimental artefact (aggregation, degradation, or conformational change) distinguishes the two forms, \( \alpha_a \) and \( \alpha_b \). Since all the antisera are raised against cytoplasmic domain peptides, glycosylation seems an unlikely possibility. Since antibodies against the shorter peptide, which is the COOH terminus, recognize the smaller subunit \( (\alpha_{5b}) \) but not the larger one \( (\alpha_{5a}) \), degradation is also an unlikely explanation. As discussed in the Results section, the three different sera react differentially with \( \alpha_a \) and \( \alpha_b \) even when all are tested in parallel (on the same integrin preparations on the same gel) under both native and denatured conditions (Figs. 2 and 3). Therefore, it is difficult to see how aggregation or conformational change could account for the results. Nonetheless, it is impossible to eliminate completely the possibility that \( \alpha_a \) and \( \alpha_b \) share the same primary sequence but differ in some other way, although this seems unlikely.

The biosynthetic data (Figs. 5 and 6) indicate that the different \( \alpha_5/\beta \)-heterodimers appear to assemble independently from a pool of precursors of \( \beta \) and the various \( \alpha \)-subunits. The presence of \( \alpha_5 \beta \)-complexes containing the precursor forms of both subunits suggests that the complexes form before completion of the carbohydrate side chains; that is, in an early Golgi or pre-Golgi compartment. Similar results have been described for a \( \beta_1 \)-integrin in melanoma cells (Cheresh and Spiro, 1987). There appears to be a large steady-state pool of \( \beta \)-precursor, particularly in mammalian cells. This pool of \( \beta \)-precursor can combine with the various \( \alpha \) precursors. When the rate of biosynthesis of \( \alpha \)-subunits is elevated by transforming growth factor-\( \beta \), the rate of processing of \( \beta \)-precursor is accelerated (Ignotz and Massague, 1986; Heino et al., 1988).

### Ligand Specificity

Turning to the question of ligand specificity, we conclude that the chicken \( \beta \)-integrin complex contains at least two fibronectin receptors and possibly more.

The argument is as follows. \( \alpha_5 \beta \), in humans has been shown to be a receptor for fibronectin, laminin, and collagens (Wayner and Carter, 1987; Wayner et al., 1988, Takada et al., 1988). In humans, \( \alpha_5 \beta \) does not bind to fibronectin affinity columns at physiological salt concentrations (Fig. 10), although it will bind in low salt (Wayner and Carter, 1987). We also observe that chicken \( \alpha_5 \beta \) does not bind to fibronectin affinity columns (Fig. 9). Comparison of the antibody blocking and affinity chromatography data suggests that human \( \alpha_5 \beta \) is a low affinity receptor for fibronectin and also a receptor for other ligands. We presume that the same is true in chickens, although formal proof requires cross-reactive \( \alpha \)-specific blocking antibodies which are not yet available.

In humans, a different fibronectin receptor was defined originally by affinity chromatography and liposome binding assays (Pytel et al., 1985a, 1986). cDNA clones isolated using antibodies to this fibronectin receptor (Argraves et al., 1987) predict an NH\(_2\)-terminal sequence which is the same as that published for human VLA-5 (Hemler et al., 1987). It has, therefore, been widely assumed that \( \alpha_5 \) is the \( \alpha \)-subunit of the original fibronectin receptor.

Which of the two \( \alpha \)-related chicken subunits is the true homologue of human \( \alpha_5 \)? The answer depends somewhat on the definition of \( \alpha_5 \). We started from published sequences (Argraves et al., 1987; Fitzgerald et al., 1987) which were obtained from cDNA clones isolated from placental or endothelial cell cDNA libraries using either polyclonal antibodies or degenerate oligonucleotides. It has been assumed that this sequence corresponds with the \( \alpha \)-subunit of the integrin heterodimer which binds to columns of FNf. Indeed, all three antipeptide antibodies used in this paper do react with the \( \alpha \)-subunit of human integrin isolated from MG63 cells on such columns (Fig. 10). If one defines the human fibronectin-binding \( \alpha \)-subunit as \( \alpha_5 \), it appears most similar in size to chicken \( \alpha_5 \) (compare Figs. 3 and 4).

However, we have been unable to demonstrate binding of chicken \( \alpha_5 \) to affinity columns of FNf (Figs. 8 and 9, and unpublished data). This may be because of its rarity on the surfaces of CEPs and in preparations of integrins from chicken embryos, or it could be a technical problem. As noted above, \( \alpha_5 \beta \), does not bind to such columns under similar conditions even though it is a fibronectin receptor by the criterion of antibody inhibition of cell adhesion to fibronectin (Wayner and Carter, 1987; Wayner et al., 1988). In contrast, \( \alpha_5 \beta \), from either fibroblasts or embryos binds well and specifically to fibronectin-affinity columns (Figs. 8 and 9). By this criterion, \( \alpha_5 \) appears functionally analogous with human \( \alpha_5 \), defined by its affinity for fibronectin.

Johansson et al. (1987a,b) have described three different fibronectin receptors isolated from different rat cell types on affinity columns of fibronectin cell-binding fragments. They appear to be integrins and to share a common \( \beta \)-subunit but have different \( \alpha \)-subunits. The \( \alpha \)-subunit from rat endothelial cells is significantly larger than the ones from hepatocytes or fibroblasts. It is conceivable that these various \( \alpha \)-subunits could include the rodent homologues of \( \alpha_5 \) and \( \alpha_6 \) in chickens. We frequently observe a doublet of two \( \alpha \)-related bands also on human cells (e.g., Fig. 4).

For the present, we think it wisest to designate the two chicken \( \alpha \)-subunits \( \alpha_5 \) and \( \alpha_6 \), to denote their cross-reactivity with antibodies to human \( \alpha_5 \). It is currently unclear which is the true structural homologue of the sequenced human \( \alpha \)-subunit and, we feel, it is also uncertain whether the published human sequence can be unambiguously related to a single fibronectin receptor.

Further research is obviously necessary to elucidate the nature of the various fibronectin receptors. In the context of the present work, the main point which we wish to make is that the chicken integrin \( \beta \)-complex contains avian homologues of two known human integrins, \( \alpha_5 \beta \) and \( \alpha_6 \). In addition, like the mammalian integrins, the avian integrins are discrete heterodimers. The original complex of proteins defined by the monoclonal antibodies, CSAT and JG22, comprises a mixture of several such heterodimers. Included in the mixture are at least two fibronectin receptors and possibly more.

Direct binding experiments with purified chicken integrins (Horwitz et al., 1985; Buck et al., 1986) and antibody blocking results with the monoclonal antibodies CSAT and JG22 (Decker et al., 1984; Tomaselli et al., 1988), both of which
react with the $\beta_1$-subunit (Buck et al., 1986), demonstrate that the $\beta$-integrins in chickens also include receptors for laminin and collagen. These could include polyspecific receptors such as $\alpha_6\beta_1$ (Wayner and Carter, 1987) or specific receptors for collagen (Wayner and Carter, 1987) or laminin (Gehlsen et al., 1988; Ignatious and Reichardt, 1988). It is not clear from our results whether chicken $\alpha_6\beta_1$ is a fibronectin-specific receptor like human $\alpha_6\beta_1$ or a polyspecific receptor like $\alpha_6\beta_2$. However, it is clear that the $\beta$-class of integrins in both humans and chickens is complex both structurally and functionally.

CEFos express, in addition, a $\beta_1$-class integrin (Fig. 7) which is functionally and immunologically related to RGD receptors isolated from a variety of human cell types (Pytela et al., 1985b, 1986; Cheresh, 1987; Cheresh and Spiro, 1987; Lawler et al., 1988). It is currently unclear whether all these human receptors are identical or merely similar. The $\beta_1$-integrin isolated from MG63 cells on RGD columns appears specific for vitronectin (Pytela et al., 1985b, 1986), whereas those isolated in similar fashion from melanoma, endothelial, or smooth muscle cells have a much wider ligand specificity, including fibronogen, von Willebrand factor, and thrombospondin, in addition to vitronectin (Cheresh, 1987; Cheresh and Spiro, 1987; Lawler et al., 1988). These $\beta_1$-integrins are not constituents of the original chicken integrin complex isolated by monoclonal antibodies, but they clearly contribute to the complexity of the integrin receptor complement on chicken cells.

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