At gastrulation in the sea urchin embryo dramatic cell adhesion changes contribute to primary mesenchyme cell ingression movements and to cell rearrangements during archenteron invagination. At ingression, quantitative adhesion assays demonstrated previously that primary mesenchyme cells (PMCs) change their affinity for neighboring cells, for a fibronectin-like substrate, and for the hyaline layer. To investigate the molecular basis for these and other differential cell affinities at gastrulation, we have identified an integrin that appears to be responsible for specific alterations in cell–substrate adhesion to laminin. During early cleavage stages blastomeres adhere poorly to laminin substrates. Around hatching there is a large increase in the ability of blastomeres to bind to laminin and this increase correlates temporally with the expression of an integrin on the basal surface all blastomeres. PMCs, after undergoing their epithelial–mesenchymal transition, have a strongly reduced affinity for laminin relative to ectoderm cells and, correspondingly, do not stain for the presence of the integrin. We identified the α integrin cDNA from Lytechinus variegatus by RT-PCR. Overlapping clones were obtained from a midgastrula cDNA library to provide a complete sequence for the integrin. The composite cDNA encoded a protein that was most similar to the α5 subgroup of vertebrate integrins, but there was not a definitive vertebrate integrin homolog. Northern blots and Western immunoblots showed that the sea urchin integrin, which we have named αSU2, is present in eggs and during all stages of development. Immunolocalization with specific polyclonal antibodies showed that αSU2 first appears on the basal cell surface of epithelia at the midblastula stage, at a time correlating with the increase in adhesive affinity for laminin. The protein remains at high levels on the basal surface of ectoderm cells but is transiently reduced or eliminated from endoderm cells during their convergent–extension movements. To confirm integrin binding specificity, αSU2 was transfected into an α-integrin-deficient CHO cell line. αSU2-expressing CHO cells bound well to isolated sea urchin basal lamina and to purified laminin. The transfected cells bound weakly or not at all to fibronectin, type I collagen, and type IV collagen. This is consistent with the hypothesis that αSU2 integrin functions by binding epithelial cells to laminin in the basal lamina. In vivo, modulation of αSU2 integrin expression correlates with critical adhesive changes during cleavage and gastrulation. Thus, this protein appears to be an important contributor to the morphogenetic rearrangements that characterize gastrulation in the sea urchin embryo. © 1999 Academic Press

Key Words: α integrin; laminin; basal lamina; sea urchin.
an affinity for hyalin and echinonectin in the apical ECM (reviewed by McClay, 1993). The loss of cell–cell adhesion is associated with the removal of cadherin from the PMC surface (Miller and McClay, 1997a), and the PMCs increase their affinity for a fibronectin-like molecule in the basal lamina (Fink and McClay, 1985). Other molecules may also be associated with this transition. For example, three integrins of unknown binding specificity have been identified in the purple urchin Stronglylocentrotus purpuratus (Marsden and Burke, 1997), and other ECM molecules have been identified in sea urchins that could be involved in this important morphogenetic event (McCarthy et al., 1987; D’Alessio et al., 1990). Details as to how these molecules function in specific changes at gastrulation are just beginning to emerge. Cadherin, for example, appears to be removed from the surface of PMCs by an endocytic mechanism (Miller and McClay, 1997a) which accounts, in part, for the rapid decrease in cell–cell adhesion observed at ingestion. There is a corresponding loss of β-catenin from adherens junctions at ingestion of PMCs (Miller and McClay, 1997b). In an effort to more completely understand the role of adhesion changes in this and in other morphogenetic events we have systematically examined known adhesion molecules and substrates for function. Here we examine how an integrin is involved in specific adhesion changes.

Integrins, the major cell surface receptors for the ECM, are dimers composed of α and β subunits that have ligand-binding specificity for various matrix components (reviewed by Hynes, 1992). A number of related vertebrate α integrins constitute a diverse family that has been classified according to sequence similarity and ligand-binding specificity (Hynes, 1992). Interactions between the extracellular matrix and the integrin receptors are known to occur during the morphogenetic movements of early development and have been shown to play a role in several model developmental systems. For example, in the chick, integrins are involved in neural crest cell migration (reviewed by Bronner-Fraser, 1994). During amphibian gastrulation, antibody injection experiments have shown that integrins are crucial for Xenopus gastrulation (reviewed by DeSimone, 1994). Several integrins have been cloned in Drosophila and found to be involved in various developmental events including germ band retraction, the attachment of muscle to tendons, and wing and eye morphogenesis (reviewed by Brown, 1993; Brown et al., 1993). Importantly, targeted gene knockouts in the mouse have shown that specific integrins are involved in early development and these integrins are largely nonredundant (Hynes, 1996).

Given the important advances in identification of adhesion molecules over the past decade, it is now imperative to characterize how these molecules contribute to specific morphogenetic events. Because of the demonstrated involvement of the ECM in sea urchin gastrulation, we investigated the role of integrins in PMC ingestion and archenteron invagination. We cloned an α integrin from Lytechinus variegatus called αSU2 integrin. αSU2 is related by sequence to Drosophila αPS2 and to the group of vertebrate α integrins which includes α5, but like αPS2, αSU2 does not have a definitive vertebrate homolog. Adhesion assays demonstrate that αSU2 binds most effectively to laminin. Adhesion assays and antibody localization studies suggest that αSU2 is associated with several morphogenetic changes in vivo. αSU2 is expressed on the basal surfaces of epithelia at the time these cells acquire an affinity for laminin. αSU2 is not present on PMCs when they ingress, and the concentration of αSU2 is modulated in invaginating endoderm. The pattern of expression and the adhesive behavior of cells suggest that αSU2 plays an important functional role in these morphogenetic movements.

**MATERIALS AND METHODS**

**Animals and Embryo Culture**

L. variegatus were obtained from the Duke Marine Laboratory in Beaufort, North Carolina, or from Sue Decker, Miami, Florida. Animals were induced to spawn by intracoelomic injection of 0.5 M KCl. Embryos were cultured in artificial seawater (pH 8.2) at 22°C.

**Embryo Cell Adhesion Assays**

Adhesion assays were performed using a quantitative method (McClay et al., 1981) that has been modified for accurate comparisons of differential affinity between two cell types (McClay and Hertzler, 1999). Microtiter plate wells were coated with substrate and subsequently treated with bovine serum albumin (40 mg/ml) to block any remaining nonspecific background binding. Control substrates either were left untreated and supported 100% binding of cells nonspecifically to the charged substrate or were pretreated with BSA alone, a treatment that eliminated binding of all cells to low background levels (0–2%). All assays included 100% and BSA-treated background binding controls. Thus any binding to the substrate coated with a matrix molecule (with sufficient BSA to blank any remaining spots on the substrate), was concluded to be binding to the substrate molecule.

To allow a comparison between two cell types on the same substrate at the same time, one group of embryos was labeled with RITC (rhodamine isothiocyanate) (Logan and McClay, 1997). Briefly, about 1 mg RITC was freshly dissolved into 50 μl dimethyl sulfoxide prior to each experiment. The culture of embryos was labeled by adding a few microliters of stock RITC to the seawater until the water turned slightly pink. Embryos accumulated the RITC for 30 min and for subsequent experiments each cell was intensely fluorescent, yet embryos so labeled developed normally if the RITC was washed out after 30 min. Cells were obtained by dissociation of embryos using nonenzymatic methods (McClay, 1986). The embryos were incubated in hyaline extraction medium (HEM) (McClay, 1986) for 5 min, incubated in calcium-free seawater (CF) for 15 min, washed with HEM, resuspended in CF, and dissociated by trituration with a 10-ml plastic syringe (without a needle attached). The cell suspension was cleared of any remaining clumps of cells by passing it twice through 20-μm Nitex mesh. PMCs were isolated from epithelial cells at the mesenchyme blastula stage by panning on wheat germ agglutinin-coated petri
Transfection of CHO Cells

An α5-integrin-deficient line of CHO cells (line B2; Schreiner et al., 1989) was transfected with αSU2 by calcium phosphate precipitation using a kit from Specialty Media, Inc. αSU2 was inserted into the mammalian expression vector pcDNA3.1 (Invitrogen, Inc.), which also contained a neomycin resistance gene. Twenty micrograms of CsCl-purified plasmid, linearized with PvuI, was added to a 100-mm plate of cells. Control transfections were performed using linearized pcDNA3.1 vector without αSU2 integrin insert. After transfection, cells were incubated with αMEM media containing 0.5 mg/ml G418, and resistant clones were picked using cloning cylinders for expansion. Transfected CHO lines were checked for expression of αSU2 by Northern blots of total RNA prepared using the Trizol kit from BRL Life Technologies.

CHO Cell Adhesion Assays

Control and adhesion-deficient B2 CHO cells, nontransfected, transfected with αSU2, or transfected with vector alone, were added to the wells (5 × 10^4 cells/well). Wells were then filled with modified Eagle's medium and sealed (McCly and Hertzler, 1998). The cells were spun down onto the substrate at 50g for 5 min at 4°C. The microtiter plates were inverted and subjected to a defined dislodgment shear force by centrifugation. Adhesion was quantified by imaging and then counting the cells remaining attached to a unit area of substrate. As a positive control, wild-type CHO cells were tested for adhesion to fibronectin; negative control assays were performed on wells coated with BSA (40 μg/ml). As before, the substrate-containing wells were posttreated with BSA to block nonspecific binding prior to addition of cells.

RT-PCR Library Screening and Sequence Analysis

Single-stranded cDNA templates for PCR were prepared from midgastrula stage L. variegatus embryos. Poly(A)^+ RNA was isolated from 100 μl packed embryos using the Pharmacia Quick-Prep Micro mRNA purification kit. First-strand cDNA was synthesized in 50-ml reaction volumes using random hexamer primers (Boehringer Mannheim) and MMLV-RT reverse transcriptase (BRL) according to the manufacturer's recommendations. Oligonucleotide primers for PCR were synthesized based on the α integrin PCR primers described by Erie et al. (1993). The forward PCR primer (αF) consisted of 5'-GGIGGAGCA/ACAG(A/C/T)TG/G(C/G)(T/C)TICT(C/T)TGGG'-3' coding for the amino acids GE(Q/L/M/LA/G)(A/S)YG. The reverse PCR primer (αR) was composed of 5'-GGIGC(C/G)GCA/C(T/G)TGCA(C/T/G)T/CAG(TC/G)CTTIC(A/G)- (A/T)ACCA/GTC-3' based on the amino acid sequence DG(F/Y)- Y/H/Q/N/K/D/E/I/E/V/I/M/A/V/I/M/G/A/A. EcoRI restriction sites were added to the ends of both primers to facilitate cloning. PCR amplifications were performed over 35 thermal cycles of 94°C for 1 min, 40°C for 1 min, and 72°C for 1 min, followed by a final 72°C incubation for 10 min. PCR products were run on a 2% agarose gel, stained with ethidium bromide, excised from the gel, and purified using the GeneClean kit (Bio101).

The PCR-amplified cDNA clone was sequenced in the Lambda ZAP using standard methods (Sambrook et al., 1989). Overlapping clones were isolated and sequenced by the chain termination method (Sanger et al., 1977) using the Sequenase II kit (Amersham). DNA and protein sequences were compiled and analyzed using the MacVector-AssemblyLign computer package (Eastman Kodak/International Biotechnologies, Inc.). Distantly related cDNA sequences were obtained by BLAST searches, pairwise with α integrin comparisons, and multiple sequence alignments were performed with the FETCH, BESTFIT, and PILEUP programs from the Genetics Computer Group software package (Devereux et al., 1984). Gap
creation penalties of 3.00 and gap extension penalties of 0.10 were used for alignments. Sequences were refined for phylogenetic analysis using MacClade (Sinauer) and trees were generated with PAUP3.1 (Swofford, 1993).

**Northern Analysis**

Poly(A)^+ RNA was prepared from eggs and various developmental stages as described above. RNA samples were fractionated on 1% agarose-formaldehyde gels and blotted to nylon membrane using standard methods (Sambrook et al., 1989). Hybridization was carried out in a buffer of 50% formamide, 400 mM NaH_2PO_4, 1 mM EDTA (pH 8.0), 5× Denhardt's solution, and 0.1% SDS. Clone 1.3 was used to generate random-primed probes as described above, and the nylon membrane was incubated overnight at 42°C. Washes were carried out as above at 65°C. The membrane was exposed to X-ray film for 3 days.

**Polyclonal Antibody Production and Staining**

A 0.9-kb BamHI fragment cut from clone 2.6, coding for the C-terminus transmembrane region and small extracellular part of the protein, was cloned into pGEX expression vector (Amrad Corp.) and used to produce a glutathione S-transferase fusion protein (GST–αSU2; see Fig. 2C). Five BALB/c female mice were given intraperitoneal injections of 10 mg GST–αSU2 fusion protein with 1:1 complete Freund's adjuvant. Three boosts of 25–50 mg GST–αSU2 were given at 2-week intervals. Preimmune and immune sera were obtained and prepared as in Harlow and Lane (1988).

For immunofluorescent localization embryos were fixed in ice-cold methanol and stored in methanol until needed. Embryos were rehydrated, washed with Tris-buffered saline, pH 7.5, containing 0.2% Tween 20 (TBST), and incubated with 1:500 dilution of αSU2 antibody in TBST containing 1% BSA for 1 h with gentle agitation. Following several washes with TBST, embryos were incubated with 1:250 dilution of anti-mouse IgG conjugated to Cy3 fluorochrome. Stained embryos were dehydrated in ethanol, mounted in methyl salicylate, and examined with a Zeiss confocal microscope. Single confocal sections were collected using a 60× oil objective and a section thickness of 1 μm.

**RESULTS**

**Sea Urchin Blastomeres Initiate Adhesion to Laminin at the Midblastula Stage**

The long-term goal of our experiments has been to dissect adhesion changes that occur during gastrulation. An improvement in our quantitative adhesion assay gave us the ability to examine quantitatively the behavior of blastomeres on candidate substrates. Embryos were dissociated at different times during early development and tested for their affinity to substrates in preliminary studies. Any differences found were studied in greater detail. The assay was set up so that we could monitor simultaneously two cell types challenged on the same substrate, under identical conditions, at the same time. In this way we predicted we would be able to detect subtle adhesive changes that might account for some of the specific morphogenetic dynamics at gastrulation. Based on those preliminary efforts, this paper reports findings of adhesion differences on a laminin substrate. The preliminary data suggested affinity changes to a laminin substrate occurred between the early blastula-stage (5 h) and the late blastula-stage (10 h) embryos. Further differences then were seen when the PMCs ingressed. These observations led to the cloning of a laminin-binding integrin and a detailed analysis of its function.

Figure 1A shows that blastomeres dissociated from early blastulae bind very poorly to laminin, while blastomeres from late blastulae bind to laminin 25- to 60-fold better under identical conditions in several independent trials. To compress a large number of experiments Fig. 1 shows results of direct comparisons of two cell types incubated in the same wells under identical conditions. One of the two cell types was prelabeled with RITC to distinguish between the two types. The results reflect the ratio of adhesion between the two cell types. In Fig. 1A, for example, if both cell types were from the same embryonic age and treated identically, the adhesion ratio was 1. In other words both cell types behaved identically. If the cells were from two different embryonic ages there was a huge difference in adhesion as reflected by an adhesion ratio that varied between 25 and 66. We conclude that blastomeres greatly increase their affinity for laminin between 5 and 10 h of development.

PMCs were then compared to ectoderm/endoderm cells for their affinity to laminin and fibronectin in adhesion assays. PMCs adhered to fibronectin about threefold better than did ectoderm in a direct comparison (Fig. 1B). In the same experiment, ectoderm adhered to laminin more than twofold better than PMCs adhered to laminin. That ectodermal adhesion to laminin was partially blocked by an antibody to laminin and adhesion of PMCs to fibronectin was blocked by an antibody to fibronectin (Fig. 1C). Thus, the data reveal that a laminin-binding cell adhesion molecule appears on blastomeres during the mid-to late blastula stage. That molecule is reduced or lost from PMCs at ingestion. The data also predict that a fibronectin-binding integrin is expressed, or at least upregulated, by PMCs at ingestion.

**Isolation of cDNA Clones for L. variegatus α Integrin**

We used PCR to isolate an α integrin fragment from midgastrula stage L. variegatus embryos. Degenerate oligonucleotide primers were synthesized based on those used previously (Erle et al., 1991). The primers amplified a 315-bp cDNA fragment that shared significant sequence similarity with other α integrins. We then screened a midgastrula stage L. variegatus expression library with the 315-bp PCR fragment to obtain overlapping clones covering the entire open reading frame. The resulting cDNA clones are indicated in Fig. 2, with the regions sequenced indicated by arrows.
Sequence Analysis of L. variegatus \(\alpha\) Integrin

Comparison of the L. variegatus \(\alpha\) integrin to other \(\alpha\) integrins in the sequence databases revealed that it clusters with Caenorhabditis elegans \(\alpha\)F54F2.1, Drosophila \(\alpha\)PS2, and the vertebrate subgroup of integrins containing \(\alpha\)IIb, \(\alpha\)5, \(\alpha\)V, and \(\alpha\)8 (Fig. 3). The phylogenetic tree generated by parsimony analysis is similar to dendrograms published elsewhere (Hynes, 1992; Whittaker and DeSimone, 1993). We have named the sea urchin integrin \(\alpha\)SU2 to reflect its sequence relationship to \(\alpha\)PS2. \(\alpha\)SU2 does not have a specific vertebrate homolog, as is also the case for the related invertebrate \(\alpha\) integrins \(\alpha\)PS2 and \(\alpha\)F54F2.1 (Wehrli et al., 1993).

Alignment of the overlapping cDNA clones produces a composite cDNA of 4262 bp, consisting of 761 bp of 5'...
untranslated region, 3123 bp of coding region, and 378 bp of 3' untranslated region. The START methionine at nucleotide position 762–764 is flanked by AGGACTATGG, corresponding to a 6/10 match for the consensus initiation sequence GCC(A/G)CCATGG (Kozak, 1987). The START methionine is followed by a signal sequence of 23 amino acids and a putative signal cleavage site at nucleotide position 830–831 (von Heijne, 1986). The first STOP codon at nucleotide position 3885–3887 produces an open reading frame of 1041 amino acids, and the mature protein of 1018 amino acids is calculated to have a molecular mass of 110,706 daltons. A large region corresponding to the extracellular domain extends to amino acid residue 982, followed by a stretch of 25 hydrophobic amino acids, which is an acceptable transmembrane domain. A short cytoplasmic domain of 31 amino acids constitutes the remainder of the sequence. A consensus site for proteolytic cleavage K/RRE/D (Bossy et al., 1991) is present at amino acid residues 876–878.

Alignment of αSU2 with other α integrins reveals a number of conserved protein domains (Fig. 4). Seven repeats characteristic of α integrins are present in the extracellular domain. Domains IV–VII each contain potential divalent cation-binding domains corresponding to at least a 11/12 match for the consensus sequence DX(D/N)X(D/N)-GXXDXXV within EF-hand calcium-binding domains (Kretsinger, 1980). Sixteen of eighteen cysteine residues are present in conserved positions throughout the extracellular domain. Eleven sites of potential N-glycosylation corresponding to the consensus NX(T/S) are present. The sequence GFFER is located in the cytoplasmic domain adjacent to the transmembrane region, a 4/5 match to the consensus GFFKR in other α integrins (Sastry and Horwitz, 1993). A potential tyrosine phosphorylation site is also present in the intracellular domain. Thus, all the major structural elements of α integrins are conserved in αSU2.

**αSU2 Is Expressed as an 11.3-kb Maternal RNA**

Northern blotting of poly(A)^+ RNA revealed that a single band of approximately 11.3 kb is present in eggs and mesenchyme blastula, midgastula, late gastrula, prism, and pluteus larval stages (Fig. 5). Comparison with ubiquitin control loadings, although not absolutely quantitative, suggests that levels of αSU2 integrin RNA remain rather constant or decrease slightly during development. Western blot data also show the protein to be present throughout development (data not shown). The protein is approx 140 kDa as blotted with the antibody made to an expressed fragment of the protein, and the calculated molecular weight of the predicted amino acid sequence alone is 110,706 kDa, suggesting that αSU2, like other α integrins, is glycosylated.

**αSU2 Protein Is Expressed in Basal Epithelia**

We next examined the localization of αSU2 protein by whole-mount immunofluorescence using an antibody generated to an expressed fragment of the protein (Fig. 6). No staining was observed in eggs or embryos up to about 5 h (not shown). Beginning in the midblastula stage (6 h), punctate
staining is first observed on the basal side of some blastomeres (Fig. 6A). By 9–10 h, in late blastula stages, there is intense staining on the basal surface of most cells in the blastoderm, especially on the thickened vegetal plate from which the PMCs and secondary mesenchyme cells (SMCs) will later ingress (Fig. 6B). There are no discontinuities in the staining pattern of the vegetal plate at this stage so we conclude that most cells, including most, if not all, PMC and SMC precursors, express the integrin at this stage. Later at PMC ingress (12 h), the basal staining pattern becomes discontinuous in the region of ingress and PMCs fail to stain for the integrin during, and following, the ingression process (Fig. 6C). Exactly when the integrin is lost in PMCs and SMCs relative to cellular ingress has not been determined in detail. Because individual PMCs ingress at times staggered over about an hour, our best estimate from the staining is that PMCs lose the integrin within an hour (perhaps less) of the ingestion event. These staining data correlate well with the data on adhesion of blastomeres to laminin. As shown initially in Fig. 1, cells from 5-h embryos adhere poorly to laminin and cells from 10-h blastulae adhere well to laminin substrates. Further, those adhesion data show that PMCs do not adhere well to laminin substrates. Together, therefore, these data suggest the hypothesis that αSU2 integrin is the receptor for laminin.

In early gastrulae (15 h), the basal side of ectodermal cells remains immunopositive but during archenteron invagination endoderm cells stain much less intensively for the integrin (Figs. 6D and 6E). In the early prism stage (Fig. 6F) the archenteron still has reduced integrin staining relative to the basal side of the ectoderm but the ectoderm/endoderm levels appear the same in plutei (Figs. 6G and 6H). These data further suggest that modulation of αSU2 integrin during gastrulation might contribute mechanistically to the convergent-extension movements that occur during this time.

**αSU2-Expressing CHO Cells Bind Laminin and Sea Urchin ECM**

Given the correlation between the early adhesion changes to laminin substrates and the αSU2 expression pattern, we sought to further test the hypothesis that cells expressing αSU2 bind to laminin. We selected a CHO cell line (B2) that previously had been shown to be largely missing α5 integrin (Schreiner et al., 1989). This cell line was transfected with αSU2 placed into a mammalian expression vector. Northern blotting of total RNA confirmed that the cell line expresses αSU2 (data not shown). Our hypothesis was that αSU2 might pair with the endogenous β integrin in the cell line and rescue binding of the mutant B2 CHO cells to laminin. To test this possibility, we challenged the transfected cells in the quantitative adhesion assay for binding to laminin, fibronectin, and other substrates. The B2 CHO mutant cell line expresses low endogenous levels of α5 integrin and is very weakly adherent to fibronectin relative to wildtype CHO cells (Schreiner et al., 1989). B2 CHO cells are also deficient in α3 and α4 integrins (Wu et al., 1995a, b).

**DISCUSSION**

**αSU2 Is Structurally Related to the α5 Integrin Subgroup**

This paper presents the first description and binding specificity of a sea urchin α integrin, which we have named...
SU2. cDNA fragments of two other sea urchin integrins have been reported in abstract form (Susan and Lennarz, 1993) and three integrins (Marsden and Burke, 1997) have been published, all in the species S. purpuratus. One of the unpublished S. purpuratus integrins appears to be a homolog of SU2 (J. Susan and R. Burke, personal communications), and we have isolated other apparent SU2 homologs from the sea urchins Eucidaris tribuloides and...

**FIG. 4.** Alignment of SU2 and human αIIb (Accession No. P08514), α5 (P08648), αV (P06756), and α8 (L36531) integrins. The signal peptide cleavage site is indicated by an arrowhead. Sequence identities across all five integrins are marked with asterisks below. The seven homologous repeat regions are indicated by boxes and roman numerals above. The four divalent cation-binding regions are indicated by horizontal lines with "11" below. Conserved cysteine residues are boxed and numbered; other cysteines are also boxed. The transmembrane region is boxed and indicated by "TM" above.

αSU2. cDNA fragments of two other sea urchin α integrins have been reported in abstract form (Susan and Lennarz, 1993) and three β integrins (Marsden and Burke, 1997) have been published, all in the species S. purpuratus. One of the unpublished S. purpuratus α integrins appears to be a homolog of αSU2 (J. Susan and R. Burke, personal communications), and we have isolated other apparent αSU2 homologs from the sea urchins Eucidaris tribuloides and...
Echinometra lacunter (unpublished data). The invertebrate integrins cloned to date are interesting from an evolutionary viewpoint, in that one group (Drosophila αPS2, C. elegans αF54F2.1, and sea urchin αSU2) clusters with the vertebrate integrins α5, αV, αIIb, and α8. However, none of these invertebrate integrins has a distinct vertebrate homolog, suggesting that the diversification of α5, αV, αIIb, and α8 occurred after the divergence of the echinoderm ancestors from the vertebrate line. Another invertebrate group that clusters together is Drosophila αPS1 and C. elegans αF54G8.3 with the vertebrate α3/6/7 group of laminin-binding integrins (Wehrli et al., 1993). Like the other members of the α5 subgroup, αSU2 contains four complete cation-binding domains, and it contains all but 2 of the 18 cysteine residues at conserved positions in this group (see Fig. 3). In contrast, αPS1, αF54G8.3, and the vertebrate α3/6/7 group contain three cation-binding domains (Wehrli et al., 1993). The cation-binding domains are thought to regulate the integrin activation state, or ligand-binding capacity, through effects on receptor conformation (reviewed by Humphries, 1996).

The calculated size the mature protein is 110,454 Da, but glycosylation at all of the 11 potential N-linked glycosylation sites would increase the size up to 140 kDa, assuming 2.5 kDa per added oligosaccharide. αSU2 contains a consensus dibasic cleavage site (K/RRE/D) near the site of heavy/light chain cleavage in other α integrins (Fig. 3), so it is likely that αSU2 is similarly processed. αSU2 contains the sequence motif GFFER in the cytoplasmic domain adjacent to the transmembrane region, which differs from the consensus GFFKR in most other α integrins. However, αPS2 also deviates from this consensus, with the sequence GFFNR (Bogaert et al., 1987), while human α9 and chick α3 have GFFRR. The conservation of this motif has been thought to imply an important functional role, but that function is unclear. Suggested possibilities include maintenance of the proper

FIG. 5. Temporal expression of αSU2 integrin message. Poly(A)⁺ RNA from eggs and embryos was probed with clone 1.3 of αSU2 integrin (upper bands). The same blot was stripped and probed with a ubiquitin probe (lower bands) to estimate gel loadings. αSU2 RNA was present in all stages examined.

FIG. 6. Localization of αSU2 integrin protein. (A) 6-h midblastula, (B) 9-h late blastula, (C) 12-h mesenchyme blastula, (D) 15-h early gastrula, (E) 18-h late gastrula, (F) 21-h prism stage, and (G, H) different optical sections from 32-h pluteus larva. Each image represents a single confocal optical section of 1 μm in thickness. Sections were selected to show a median plane through the embryo. Scale bar, 20 μm.
insertion in the membrane, subunit association, cytoplasmic protein binding site, or a role in receptor activation through conformational change (Sastry and Horvitz, 1993; Williams et al., 1994).

**αSU2 Is a Laminin-Binding Integrin**

Fink and McClay (1985) demonstrated differential adhesion of sea urchin blastomeres to fibronectin. Primary mesenchyme cells were observed experimentally to increase their affinity toward fibronectin at ingestion. Given its structural relationship to the α5 subgroup, we initially expected that αSU2 might bind to fibronectin. Expression and adhesion data instead support the hypothesis that αSU2 is an integrin that binds to laminin. This conclusion is reinforced in that PMCs failed to have a strong affinity to laminin and did not stain with the antibody to αSU2. Epithelial adhesiveness to laminin occurs coincident with αSU2 expression on the basal side of the epithelial cells. Finally, laminin-binding specificity was directly demonstrated by affinity differences in transfected cells. αSU2-expressing CHO cells bound very weakly to fibronectin and not at all to FNfn7-10, suggesting that αSU2 does not bind to the RGD binding site of fibronectin. In contrast, αSU2-expressing cells bound strongly to laminin relative to nontransfected controls. Support of adhesion even at low laminin substrate concentrations and relatively high dislodgment forces is consistent with the hypothesis that αSU2 is a laminin-binding integrin. It remains formally possible that αSU2 integrin is never expressed on PMC and SMC precursors. However, the vegetal plate staining data of premesenchyme blastulae do not support that notion. A fate map of cells at the late blastula stage shows that most of the central vegetal plate is occupied by PMC and SMC precursors (Ruffins and Ettensohn, 1996). If these cells had not acquired the αSU2 integrin, there should have been a large gap in staining in the vegetal plate. This was not seen. We conclude that αSU2 integrin contributes to epithelial cell-substrate adhesion and that changes in expression of that molecule are involved in the epithelial-mesenchymal conversion that characterizes PMCs and SMCs.

**αSU2 Is an Epithelial Integrin**

The antibody localization of αSU2 on the basal surface of epithelial cells and the cell binding data suggest that this integrin functions in adhesion of epithelia to the basal lamina. The earliest detection of the protein at the cell surface by immunolocalization is a punctate staining pattern on the basal side of epithelial cells at the midblastula stage. Prior to the midblastula stage the blastomeres adhere preferentially to the extraembryonic matrix, hyaline. As the embryo approaches gastrulation an affinity for the basal lamina becomes predominant (Fink and McClay, 1985; Burdsal et al., 1991). The immunofluorescence data and the adhesion data are in agreement in the studies performed. They suggest that prior to the midblastula stage blastomeres do not adhere to laminin and bind only weakly to the basal lamina. After the appearance of αSU2 on the basal cell surface, the blastomeres display a dramatic increase in their affinity to laminin. αSU2 may therefore serve as the primary receptor binding the blastula-stage cells to the basal lamina.

αSU2 expression changes during primary mesenchyme cell ingestion and during archenteron invagination. Before PMC ingestion, αSU2 integrin is present as a continuous layer along the basal surface of the epithelium, but is not present on ingressing PMCs, nor is it later present on delaminated secondary mesenchyme cells. It is not clear what becomes of αSU2 in the PMCs and SMCs, since no staining suggestive of endocytosis...
is present after their ingression. PMCs demonstrate an increased affinity for fibronectin after ingression, and they have a low affinity for laminin. These data further support the notion that αSU2 is an epithelial integrin in that cells that have undergone an epithelial-mesenchymal transition fail to express αSU2. By contrast, sea urchin βG integrin is reported to be upregulated in PMCs after their ingression (Marsden and Burke, 1997) and may serve as a partner for a fibronectin-binding α integrin, though no functional data are published for that integrin.

A similar change in expression of integrins during an epithelial-mesenchymal conversion has been documented in metastatic cells undergoing invasive behavior. For example, the collagen/laminin-binding α2β1 integrin is normally expressed at high levels in mammary epithelia (reviewed by Ziober et al., 1996), while mammary carcinoma cells express low or undetectable levels of α2β1 integrin. Transfection of α2β1 into a α2β1-negative breast cancer cell line causes a strong change from malignant phenotype to a contact-inhibited, less invasive, epithelial phenotype (Zutter et al., 1995). Other integrins are upregulated during metastatic behavior. Malignant melanoma cells express elevated levels of αVβ3 integrin, for example, and experimental transfection of αV into αV-deficient cells causes increased adherence and invasive behavior (reviewed by Marshall and Hart, 1996). In contrast to cancer cells, the change in integrin expression during PM ingression appears to be a normal adhesive change as part of a morphogenetic movement. Thus, the differential expression or activation of integrins likely is a general feature of epithelial-mesenchymal conversions during morphogenesis.

During invagination of the archenteron presumptive endoderm cells display a reduced level of αSU2 staining relative to ectoderm. The staining returns at the prism stage when gastrulation is complete. These observations are consistent with the notion that αSU2 integrin expression is reduced to facilitate the convergent-extension movements of the archenteron. During convergence-extension the endoderm cells slide past one another and take up new positions. A potential consequence of reduced αSU2 integrin expression during these morphogenetic movements is a reduced affinity of endoderm for the basal lamina. Unfortunately we have been unable to directly test this hypothesis because methods for separating endoderm and ectoderm at this stage are unavailable (there is a method for separation of ectoderm from endoderm but only later, when convergence-extension is complete; McClay and Marchase, 1979).

In other studies it has been observed that archenteron invagination can be disrupted by laminin peptides YIGSR and IKVAV, prompting the suggestion that adhesion to laminin is necessary for this process (Hawkins et al., 1995). Our data are in contrast to that study since αSU2 expression on endoderm cells appears reduced in the archenteron during its elongation. Instead, this reduced level of integrin expression is consistent with hypotheses that cell rearrangements during archenteron extension might occur more readily if substrate binding were relaxed (Ettensohn, 1985b; Hardin, 1989). Other integrins appear to be differentially expressed in the archenteron as well. At least one of the sea urchin β integrins (βG) appears to be preferentially expressed in the archenteron (Marsden and Burke, 1997). It will be necessary to determine the expression pattern and ligand specificity of βG integrin and a larger complement of sea urchin integrins before their role in archenteron morphogenesis and PMC ingression can be understood in detail.

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