Chapter 3

Modulation of $\alpha_2\beta_1$ integrin changes during mammary gland development by $\beta$-estradiol*

3.1 Introduction

As discussed in the introductory chapter, several in vitro studies using cultures of mammary epithelial cells have demonstrated that the extracellular matrix influence cellular functions [Bissell et al., 1982; Bissell et al., 1985; Li et al., 1987; Barcellos – Hoff et al., 1989]. Although no direct evidence for the influence of the matrix on tissue specific gene expression in vivo exists, studies on changes in the matrix metalloproteinases (MMPs) in mammary gland at different stages of development indicated that the extracellular matrix can influence tissue – specific functions [Alexander and Werb, 1991; Birkedel – Hansen et al., 1993; Mignatti et al., 1996; Talhouk et al., 1992; Ambili et al., 1997]. In vivo, modulation of cell - matrix interactions occurs through changes in cell surface molecules involved in mediating cell – matrix interactions. As reviewed in detail in the first chapter, the integrins are a large family of transmembrane proteins which form heterodimers that mediate cell – ECM and cell – cell interactions [Hynes, 1987; Buck and Horwitz, 1987]. In vitro studies have shown that consequent on ECM - integrin interactions, a cascade of events occur intracellularly, leading to tissue – specific gene expression [Hynes, 1992; Burridge et al., 1992].

$\alpha_2\beta_1$ integrin has been identified as the major collagen receptor in the mammary gland and appears to play an important role in mammary gland morphogenesis and tissue – specific gene expression. Since the mammary gland undergoes developmental changes throughout adult life, changes in integrins which mediate the cell - matrix interaction was examined. The molecular mechanism of alteration in cell - matrix interactions was studied by examining the temporal pattern

of expression of $\alpha_2\beta_1$ integrin in rat mammary gland at different stages of development. The results presented in this chapter indicate that the changes in $\alpha_2\beta_1$ integrin during mammary gland development are modulated by $\beta$-estradiol.

### 3.2 Materials and Methods

Mammary epithelial cells were isolated from midpregnant (12 days of gestation) rats by collagenase digestion and the attachment of the epithelial cells to collagen I and to the CNBr fragments of Col I was assessed. Mammary gland tissue was extracted with hypotonic buffer and the Col I binding proteins from plasma membrane were isolated by affinity chromatography over Col I - sepharose column. The proteins eluted from the column were characterized by SDS – PAGE and immunoblotting using specific antibodies. Level of Col I binding proteins from mammary glands of different developmental stages was assessed by dot blot assay and the quantitation of $\alpha_2\beta_1$ integrin was done by ELISA. The localization of the integrin in intact tissue was done by immunocytochemistry. The effect of $\beta$ - estradiol on the production of $\alpha_2\beta_1$ integrin was examined by supplementing primary cultures of rat mammary epithelial cells with $\beta$ - estradiol in a medium containing $^{35}$[S] - methionine. The effect of the hormone in vivo was determined by extracting plasma membrane proteins from hormone administered rats and by determining the level of the integrin by ELISA and immunocytochemistry. The details of the various methods have been given in Chapter 2.

### 3.3 Results

#### 3.3.1 Attachment of rat mammary epithelial cells to Col I and to the CNBr fragments of Col I

In order to examine the cellular specificity of interaction with collagen, attachment of rat mammary epithelial cells to Col I substratum and to the CNBr fragments of Col I was assessed. Mammary epithelial cells isolated from midpregnant rats were allowed to attach on tissue culture plates passively coated with different concentrations of Col I and to the different CNBr fragments of Col I, and the attachment of cells at different time intervals was measured. The results are given in Figs. 3.1 and 3.2. There was a concentration dependent increase in the attachment of cells and showed maximum attachment at a concentration of 10 $\mu$g indicating a saturation kinetics (Fig. 3.1). The maximum attachment was obtained within 60 min. of incubation (Fig. 3.2).
Rat mammary epithelial cells (1 x 10^6 cells/ml) were seeded on 35 mm plastic petri dishes passively coated with different concentrations of Col I. Cells were allowed to attach at 37°C for 1 hr. Attached and unattached cells were separated and lysed in 0.1 % triton X-100 and the activity of β-hexosaminidase was determined in the cell lysate as described in the methods. Percentage attachment was calculated from the enzyme activity. Values given are the average of 4 experiments (± SD).

Rat mammary epithelial cells (1 x 10^6 cells/ml) were seeded on 35 mm plastic petri dishes passively coated with Col I (10 µg/ml). Cells were allowed to attach at 37°C for different time intervals. Attached and unattached cells were separated and lysed in 0.1 % triton X-100 and the activity of β-hexosaminidase was determined in the cell lysate as described in the methods. Percentage attachment was calculated from the enzyme activity. Values given are the average of 4 experiments (± SD).
The cells also attached to different CNBr fragments of collagen. Results presented in Fig. 3.3 indicate that all the five CNBr fragments promote attachment. The maximum attachment was given by CB3 and CB4 fragments, which are high molecular weight peptides. The low molecular weight peptides CB1, CB2 and CB5 fragments also showed fairly high attachment promoting activity, indicating that the Col I molecule possesses multiple binding sites for the mammary epithelial cells.

**Fig. 3.3 Attachment of rat mammary epithelial cells to the different CNBr fragments of Col I**

![Graph showing attachment of rat mammary epithelial cells to different CNBr fragments of Col I](image)

Rat mammary epithelial cells (1x 10^6 cells/ml) were seeded on 35 mm plastic petri dishes passively coated with equal concentrations of CNBr fragments of Col I (50 µg/ml) and allowed to attach at 37°C for 1 hr. Attached and unattached cells were separated and percentage attachment was determined. Values given are the average of 4 experiments (± SD).

### 3.3.2 Binding of plasma membrane proteins from mammary gland to ^125^I-labeled CNBr fragments of Col I

The specificity of interaction of mammary epithelial cells with Col I was further studied by dot blot assay using ^125^I-labeled CNBr fragments of Col I, and the results are given in Fig. 3.4. The plasma membrane proteins from mammary gland bound to the different CNBr fragments, both the low molecular weight and high molecular weight peptides. Binding was found to be maximum to the fragment CB3.
Nitrocellulose membranes were coated with equal amounts of plasma membrane proteins (50 µg/ml) isolated from rat mammary gland. The radiolabeled CB1, CB3 and CB4 (~3000 cpm/ml) were allowed to bind to the membranes at 37°C for 1 hr. The discs were washed with TBS thrice and the radioactivity bound to the membrane and the unbound activity were measured. Percentage of the ligands bound was calculated. Values given are the average of 4 experiments (± SD).

3.3.3 Isolation and characterization of Col I binding proteins from rat mammary gland

In order to identify the Col I binding proteins in mammary gland, plasma membrane extract of the midpregnant rat mammary gland was subjected to affinity chromatography on Col I - sepharose and the bound proteins were eluted using 20mM EDTA. The elution profile is represented in Fig. 3.5. The proteins eluted by EDTA were characterized by electrophoresis. Two bands possessing a molecular size of 160 kDa and 130 kDa appeared on SDS – PAGE under reducing conditions, as represented in Fig. 3.6. As the molecular size of these peptides appear to correspond to that of α₂ and β₁ chains of integrins and since α₂β₁ is known to be a collagen receptor, it was confirmed by immunoblotting. Western blotting followed by detection with specific antibodies to α₂ and β₁ subunits, gave positive reaction further confirming the presence of α₂β₁ integrin (Fig. 3.7). The proteins eluted at acid pH were not processed further.
The plasma membrane extract of rat mammary gland was subjected to affinity chromatography over Col I- Sepharose column. The unbound proteins were washed with 0.05M Tris/ HCl buffer. The bound proteins were eluted with 20mM EDTA and then with 0.2M glycine / HCl. 2 ml fractions were collected and the OD was measured at 280 nm. The fractions collected with 20mM EDTA were pooled together, dialysed, concentrated and subjected to SDS – PAGE analysis.

Plasma membrane proteins isolated from rat mammary gland by affinity chromatography over Col I - Sepharose 4B followed by elution with EDTA were characterized by SDS-PAGE analysis on 7.5% gel under reducing conditions and developed by silver staining. Myosin (205 kDa), β-galactosidase (116 kDa) and bovine albumin (66kDa) were used as markers.
Fig. 3.7 Immunoblotting of $\alpha_2\beta_1$ integrin from rat mammary gland

![Immunoblot of collagen binding proteins](image)

Purified collagen binding protein was subjected to SDS-PAGE followed by western blotting and located using specific antibodies. Immunoblot of collagen binding proteins was probed with anti $\alpha_2$ and anti $\beta_1$ antibodies. Myosin (205 kDa), $\beta$-galactosidase (116 kDa), and bovine albumin (66kDa) were used as markers.

Fig. 3.8 Binding of $^{125}\text{I}$-labeled $\alpha_2\beta_1$ integrin to Col I and Col IV- Dot blot Assay

![Dot blot Assay](image)

The nitrocellulose membrane were coated with 10µg/ml of Col I and Col IV. The radiolabeled $\alpha_2\beta_1$ integrin (3000 cpm/ml) isolated from the mammary gland was allowed to bind to the membranes at 37°C for 1 hr. The discs were washed with TBS thrice and the radioactivity bound to the membrane and the unbound activity were measured. Percentage of the ligands bound was calculated. Values given are the average of 4 experiments ($\pm$ SD).
The binding of $\alpha_2 \beta_1$ integrin isolated from rat mammary gland to Col I and Col IV was confirmed by dot blot assay using radioiodinated $\alpha_2 \beta_1$ integrin and the results are given in Fig. 3.8. The binding was found to be high on both Col I and Col IV, though it was slightly higher to Col I substratum.

3.3.4 Changes in $\alpha_2 \beta_1$ integrin expression in the mammary gland at different stages of development

Since the mammary gland undergoes developmental changes during adult life, the interaction of mammary epithelial cells with the ECM may also change. So, the changes occurring in the binding of iodinated Col I to plasma membrane proteins from different stages of mammary gland was studied. The yield and purity of plasma membrane preparation from different stages were checked by assay of the enzyme 5'-nucleotidase. The results are given in Fig. 3.9 and in Table 3.1. The enzyme activity remained high and almost the same during the gestational and lactating stages. But it was slightly lower in the case of virgin tissue and was comparable to the involuting stages.

**Fig. 3.9 Assay of 5'-nucleotidase in the mammary gland tissues from different stages of development**

Plasma membrane of mammary gland from different stages of development were prepared and the 5'-nucleotidase assay was done as described in the methods. Enzyme activity is expressed as units/mg protein. Values given are the average of 5 experiments ($\pm$ SD).
Table 3.1

Yeild of plasma membrane from mammary gland tissues

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Activity of 5' nucleotidase (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin</td>
<td>34.6</td>
</tr>
<tr>
<td>Midpregnant</td>
<td>39.8</td>
</tr>
<tr>
<td>Lactating</td>
<td>32.4</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; involuting day</td>
<td>33</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; involuting day</td>
<td>34.5</td>
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The binding of $^{125}$[I] - Col I to plasma membrane proteins from different stages showed a different pattern. The binding was maximum in the case of midpregnant stage, showing nearly 20% increase compared to virgin stage, which also showed high binding affinity. But the binding of $^{125}$[I] - Col I to the membrane proteins from the lactating stage was found to be minimum. But the binding of $^{125}$[I] - Col I to the plasma membrane proteins of involuting stage was found to be higher than that of the lactating stage. These results are presented in Fig. 3.10.

Fig. 3.10  Binding of $^{125}$[I] - Col I to plasma membrane proteins from rat mammary gland from different stages of ontogeny – Dot blot assay

Nitrocellulose discs were coated with 20 µg/ml plasma membrane proteins from different stages of development and $^{125}$[I] - Col I was allowed to bind for 1 hr at 37°C. 3% BSA was taken as control. V - virgin, M - midpregnant, L - lactating, 2I - 2<sup>nd</sup> involuting day and 6I- 6<sup>th</sup> involuting day. Radioactivity bound to the membrane after washing with TBS, and the unbound activity was measured. Percentage of ligand bound was calculated. Values given as percentage of virgin are the average of 4 experiments (± SD).
Since, there was a change in the level of the plasma membrane proteins that bound to collagen, during mammary gland ontogeny, the change occurring in the level and distribution of the $\alpha_2\beta_1$ integrin was studied. Mammary gland tissues from varying stages of development, viz., virgin, midpregnant, lactating and involuting stages were extracted and analyzed by ELISA using antibodies against $\alpha_2$ integrin (Fig. 3.11). The virgin tissue was found to express a considerable amount of $\alpha_2\beta_1$ integrin which increased significantly (nearly 20%) in the midpregnant stage. The level of the integrin during the estrous and proestrous stages of the virgin rat mammary gland was also determined by ELISA and the results are given in Fig. 3.12. The results showed that there was only a slight increase in the integrin level during the estrous stage, compared to the proestrous. The level of the integrin in the virgin tissue is taken as the average of these two sets of values.

**Fig. 3.11 Changes in $\alpha_2$ integrin level in mammary gland during different stages of development**

![Graph showing changes in $\alpha_2$ integrin level](image)

Equal amounts of plasma membrane proteins (20 µg/ml) from virgin (V), midpregnant (M), lactating (L), 2nd involuting day (2 INV) and 6th involuting day (6 INV) stages were coated on multiwell plates and analysed by ELISA using anti $\alpha_2$ antibody. OD units were expressed as percentage of that of virgin tissue. Values given are the average of 5 experiments (± SD). P< 0.01 when the lactating stage was compared with virgin, P< 0.05 when the other stages were compared with virgin.
Fig. 3.12 Level of $\alpha_2\beta_1$ integrin in virgin mammary gland from proestrus and estrous stages

![Graph showing level of $\alpha_2\beta_1$ integrin in virgin mammary gland from proestrus and estrous stages.]

Equal amounts of plasma membrane proteins from the proestrus (□) and estrous (■) stages of the virgin rat mammary gland were coated on multiwell plates and ELISA was done to quantitate $\alpha_2\beta_1$ integrin using specific antibodies. Values given are the average of 5 experiments (± SD).

The expression of $\alpha_2\beta_1$ integrin during pregnancy was found to remain high up to later stages as evidenced by ELISA, the results of which are given in Fig. 3.13. But the level was found to be very low on the day of parturition. As involution sets in, the integrin reappears gradually, attaining a level similar to that of the virgin tissue. Thus it appears that there was an upregulation of the $\alpha_2\beta_1$ integrin in pregnancy, while it was significantly down regulated in the lactating phase.

3.3.5 Localization of the $\alpha_2\beta_1$ integrin by immunocytochemical analysis

The changes, if any, in the distribution pattern of $\alpha_2\beta_1$ integrin as the mammary gland undergoes developmental changes were also studied by immunocytochemical analysis. Tissue sections from mammary gland at different stages were stained with antibody against $\alpha_2$ integrin. Positive reaction was indicated by brown staining. $\alpha_2$ integrin was found to be expressed by virgin, midpregnant and involuting mammary glands, while the lactating gland
did not show positive reaction (Fig. 3.14). For the virgin tissue, expression was moderate and the distribution was found to be mainly towards the luminal area of epithelial cells along the ducts and the ductules of the gland. In the midpregnant stage also, a similar expression pattern was seen. It was followed by a down regulation of the integrin in the lactating stage. But, as involution sets in, the integrin reappeared and the intensity of staining was mild. No staining was seen in the control sections untreated with primary antibody, indicating that the reaction observed was not non-specific.

3.3.6 Effect of estradiol on the production of $\alpha_2\beta_1$ integrin by isolated mammary epithelial cells in culture

In order to study whether the altered expression of $\alpha_2\beta_1$ integrin in the mammary gland was due to hormonal control, the effect of estradiol on the $\alpha_2\beta_1$ integrin production by the mammary epithelial cells was studied. Mammary
Legends to figure

Fig. 3.14 Immunocytochemical localization of $\alpha_2$ integrin

Virgin (B), midpregnant (C), lactating (D), 2nd day involuting (E) and 6th day involuting (F) mammary tissue sections were stained with antibody against $\alpha_2$ integrin and counterstained with Mayer's hematoxylin as described in the methods. Midpregnant tissue untreated with primary antibody served as control (A). Arrows indicate regions of positive staining. Bar = 100 µm.
Mammary epithelial cells from virgin (⧫–⧫) and lactating tissues (■–■) were metabolically labeled with $^{35}$S-methionine (20μCi/ml) and treated with β-estradiol ($10^{-6}$ to $10^{-4}$ M) for 12 hrs. The cell layer was extracted in detergent buffer and $\alpha_2\beta_1$ integrin was quantified as described in the text. Cells from virgin and lactating tissues untreated with estradiol served as control.

epithelial cells from virgin and lactating tissues in culture were treated with different concentrations of the hormone ($10^{-6}$ to $10^{-4}$M) in $^{35}$S-methionine containing medium. The medium and the cell layer were collected after 12 hrs. The detergent extract of the cell layer was subjected to affinity chromatography on Col I-sepharose and the radioactivity incorporated into $\alpha_2\beta_1$ integrin was determined. There was an increase in the amount of the integrin synthesized both by the virgin tissue and the lactating tissue with increase in concentration of the hormone as presented in Fig. 3.15. The amount of $\alpha_2\beta_1$ integrin in the cells from the virgin stage tissue increased with the increase in concentration of the hormone. Though the integrin level of the lactating stage was very low compared with the virgin stage, after estradiol treatment of cells, there was an increase in the level of integrin, with increase in concentration of the hormone.

### 3.3.7 Effect of estradiol treatment in vivo on $\alpha_2\beta_1$ integrin in mammary gland

In order to study the effect of estradiol in vivo, virgin and lactating rats were administered estradiol and the plasma membrane extracts were prepared and ELISA was done for the quantitation of $\alpha_2\beta_1$ integrin using specific antibody against the integrin and the results are given in Fig. 3.16. In both cases, after estradiol
Fig. 3.16  Effect of β-estradiol treatment in vivo on α₂β₁ integrin production in mammary gland

Plasma membrane extracts prepared from virgin (A) and lactating (B) tissues after administration of estradiol (0.6 mg/kg bodyweight for 3 days) and used for experiment. ELISA was done using specific antibodies against the integrin as described in the methods. OD units were expressed as % of that of virgin tissue. Values given are the average of 4 experiments (± SD). Virgin stage after estradiol treatment when compared with control, P< 0.05; lactating stage after treatment when compared with control, P< 0.01. (☐ untreated, ■ treated)

administration, the level of the integrin was elevated. Though the level of the integrin in the normal lactating gland was very low, after estradiol treatment, it was elevated by about four times the untreated control levels.

3.3.8 Immunocytochemical analysis of estradiol treated tissue

The expression of α₂β₁ integrin on treatment with β-estradiol was also tested by immunocytochemical analysis. Sections of both virgin and lactating tissues after estradiol administration were subjected to immunocytochemical analysis. The results of this experiment are presented as Fig. 3.17. The luminal side of cells in each acini, the ducts and ductules of the gland showed positive staining. The normal lactating gland did not show significant reaction, but after estradiol treatment, the staining became intense. Immunopositivity was found to be towards the luminal side of cells in acini, the ducts and myoepithelial cells also showed positive staining. While β-estradiol administration caused only a slight increase in intensity of staining in virgin tissues, it resulted in intense positive reaction in lactating tissues.
Legends to figure

Fig. 3.17  Effect of estradiol administration on $\alpha_2$ integrin distribution in mammary gland

Animals were administered $\beta$-estradiol and mammary gland tissues were collected. Tissue sections from virgin (B) and lactating (D) animals administered estradiol were stained with antibody against the $\alpha_2$ integrin and counterstained with Mayer’s hematoxylin as described in the methods. Sections of tissues from untreated virgin (A) and lactating (C) glands served as control. Arrows indicate regions of positive staining. Bar = 100 µm.
3.4 Discussion

Interaction of cells with the extracellular matrix is mediated by cell surface receptors for ECM. The major cell surface receptors are the integrin family of transmembrane proteins. Normal mammary cells express the different subunits of the integrin superfamily for mediating the cell – ECM or cell – BM interactions. These are mainly $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_6$, $\beta_1$, and $\beta_4$ subunits. In the mammary gland, the interaction between epithelial cells and collagen mediated by integrin is especially important, as evidenced by several in vitro studies [Streuli et al., 1991; Keely et al., 1995b; Klinowska et al., 1999]. The results presented here provide further evidence that the $\alpha_2\beta_1$ integrin is the major receptor for collagen in mammary gland. This is confirmed by SDS – PAGE analysis and immunoblotting. The integrin appeared on SDS – PAGE as 160/130 kDa bands. The presence of these subunits was further confirmed by immunoblotting using specific antibodies. In the virgin mammary gland, the $\alpha_2$ integrin was seen towards the luminal side of epithelial cells in the acini and also in the duct system. During gestation stage, when proliferation of epithelial cells occurs, there is a significant increase in $\alpha_2\beta_1$ integrin level which appeared at the cell – matrix contact sites and the level remained high till the end of gestational stage. This may promote cell – matrix interactions and contribute to tissue remodeling during mammary gland development. $\alpha_2\beta_1$ integrin was reported to be associated with orderly and regulated proliferation of epithelial cells including the ducts and the ductules of normal breast [Zutter and Santoro, 1990]. The $\alpha_2\beta_1$ integrin was also found to be present on the basal, lateral and apical surfaces of the mammary epithelium [Keely et al., 1995b]. Our results show that in the lactating tissue, where epithelia showed a structural change and tissue – specific gene expression such as milk production is maintained, $\alpha_4$ integrin level is very low. It appears that there is a down regulation of the $\alpha_2$ integrin during the lactating stage.

The down regulation of the integrin during lactation is not due to change in epithelial cell mass. The assay of 5' – nucleotidase which is a marker for the cell membrane was done using plasma membrane isolated from different stages of mammary gland at different stages of ontogeny. The enzyme activity was found to be the same both in the gestational and lactating stage indicating that the difference in the $\alpha_2\beta_1$ integrin levels observed here was not due to a difference in the yield of the plasma membrane preparation. Thus it appears that the changes observed in the level of $\alpha_2\beta_1$ integrin in mammary gland during different stages of ontogeny are not any apparent effect.
From our results, it is evident that a high level of $\alpha_2\beta_1$ integrin is maintained during gestation, when epithelial proliferation and glandular structure formation occur in the mammary gland. The down regulation of the integrin in the lactating stage may be a regulatory mechanism to arrest the morphogenetic event. A decrease in $\alpha_2\beta_1$ integrin level disrupts the ability of mammary epithelial cells to organize into three dimensional collagen gels, indicating that $\alpha_2\beta_1$ integrin plays a crucial role in collagen induced morphogenesis [Keely et al., 1995a]. The normal development and ductal morphogenesis of the mammary gland depend on functional $\beta_1$ integrins which permits contacts with the ECM and with laminin in particular [Klinowska et al., 1999]. It is also suggested that cell growth, survival and morphogenesis of acinar structures in normal tissues are integrin dependent and loss of proper integrin mediated cell – ECM interactions may be critical to breast tumor formation [Howlett et al., 1995]. The results presented here provide further evidence in support of a critical role for the interaction of cells with ECM in vivo in mammary epithelial function. Changes in the level of $\alpha_2\beta_1$ integrin not only affect epithelial – basement membrane interactions but also may influence intracellular events.

The increase in $\alpha_2\beta_1$ integrin during pregnancy, may be proliferation associated, but during lactation, there is a down regulation of the integrin, though the epithelial mass remains the same. This may also be due to the action of hormones, since the development of the mammary epithelium and milk production during the later stages of pregnancy and in lactation are under hormonal control. The normal development of the mammary gland was found to be partly under control of interaction between gonadotrophic hormone and estrogen [Feldman et al., 1993]. Results on the production and distribution of $\alpha_2\beta_1$ integrin presented here suggest that hormones influence cell – matrix interactions as well. It appears that the production and distribution of $\alpha_2\beta_1$ integrin is modulated by estrogen. This conclusion is based on the following observations. (a) In mammary glands from midpregnant rats, which is under estrogen stress, the level of $\alpha_2\beta_1$ integrin is elevated (b) In lactating tissue, which is relieved from estrogen stress, the level of the $\alpha_2\beta_1$ integrin is decreased (c) Administration of $\beta$-estradiol caused an increase in the level of the integrin. Both in the virgin and in the lactating stage, the level of the integrin was significantly enhanced after estradiol treatment. The distribution pattern of the $\alpha_2\beta_1$ integrin in lactating glands treated with estradiol was also similar to that of virgin and pregnant stages. (d) In vitro experiments by supplementing estradiol to primary cultures of mammary epithelial cells also demonstrated that the $\alpha_2\beta_1$ integrin production by cells from both the virgin and lactating tissues is increased in the presence of estradiol.
Although these experiments do not indicate how estrogen affects the production and distribution of $\alpha_2\beta_1$ integrin in mammary gland, its enhanced production by primary cultures of mammary epithelial cells on treatment with estradiol indicates that estradiol effect is not a systemic one. The influence of hormones on the cell surface receptors and changes in tissue specific functions have been reported in different systems [Yoshimura et al., 1998; Sillem et al., 1997; Sonohara et al., 1998]. The addition of estradiol and progesterone to cultured stromal cells of the human endometrium in the early proliferative phase increased the expression of $\beta_1$ integrins in vitro [Yoshimura et al., 1998]. It has been suggested that estrogen enhances $\alpha, \beta_3$ expression by avian osteoclast precursors through stabilization of $\beta_1$ integrin mRNA.

It appears that steroid hormones, particularly estrogen has an important role in regulating cell - matrix interactions in mammary gland. It has been reported that steroid hormones apart from their intracellular effects influence composition of the extracellular matrix. The data available in the literature indicates that the modulation of cell - matrix interactions by steroid hormones can be through different mechanisms. This may be due to a change in the composition of ECM components. Change in the composition of matrix can be caused by steroid hormones by influencing the intracellular events involved in the production of these components. [Feng et al., 1995; Woodward et al., 2001]. Change in composition of ECM can also be caused by modulation of activity of enzymes such as MMPs which are involved in degradation of ECM components. A role for $\beta$ - estradiol in mediating matrix remodeling by regulation of MMPs involved in mammary gland involution has also been demonstrated [Ambili et al., 1998; Tonner et al., 2000]. Estradiol acts locally in the mammary gland, stimulating DNA synthesis and promoting duct formation. This is mediated by an estrogen receptor ER – both ER$\alpha$ and ER$\beta$. It is expressed in both epithelial and stromal cells, so that the morphogenetic effects of estrogen could be mediated via epithelial or stromal ER. During gestation, the proliferation of epithelial mass is due to this hormonal influence [Cunha et al., 2000].

Yet another mechanism which may be involved in mediating cell - ECM interactions by steroid hormones is through the modulation of cell surface receptors involved in mediating interaction of cells with ECM components. The present data give evidence for the hormonal modulation of cell – ECM interactions, as a result of changes occurring in the cell surface receptors for matrix proteins as shown in the case of $\alpha_2\beta_1$ integrin expression in mammary gland.
In vitro studies using mammary epithelial cells have suggested that ECM influence cellular activity and expression of tissue specific functions. Although ECM is known to have a structural function in mammary gland in vivo, no direct experimental evidence exists in support of a role for ECM in influencing the expression of tissue specific genes in vivo. However, there are indications of changes in the composition and distribution of ECM during different stages of mammary gland development [Keely et al., 1995b]. Experimental data also exist indicating the occurrence of matrix responsive elements in the tissue specific genes such as those of α-casein in the mammary gland. Based on the results on the expression of MMPs which are involved in the degradation of ECM components and control of cellular microenvironment and expression of TIMP in vivo, a correlation between expression of tissue specific genes and changes in the mechanisms involved in remodeling of ECM in vivo has been demonstrated [Talhouk et al., 1992b]. Results presented in this chapter on α₂β₁ integrin which is a major receptor for collagen in the mammary gland during different stages of development provide further evidence in support of the influence of ECM on cellular activity in vivo. During gestational stage where epithelial proliferation and remodeling of ECM occur, there is an upregulation of α₂β₁ integrin which probably would promote cell-ECM interaction. When it enters the lactational phase where the cell-ECM ratio is maintained constant and also where steady and sustained expression of tissue specific genes occur, there is a downregulation of the integrin.