

**P052** Interplay between integrin subunits in signaling  
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Integrins-type cell adhesion receptors transmit bidirectional signals across the plasma membrane. We have studied the mechanisms of inside-out and outside-in signaling in collagen receptor  $\alpha 2\beta 1$  integrin.

We propose that glutamate 336 (E336) in the ligand binding  $\alpha 2I$  domain acts as an “intrinsic” ligand for the  $\beta 1I$  domain, leading to a conformational change and signaling. In a flow cytometry based binding assay, the mutant  $\alpha 2_{E336A}\beta 1$  expressed on CHO cells showed remarkably low binding to collagen. However, the mutation appeared not to prevent the TPA related increase in  $\alpha 2_{E336A}\beta 1$  adhesion. Integrin cluster formation was not affected in  $\alpha 2_{E336A}\beta 1$  cells. Instead, the mutation E336A prevented a characteristic  $\alpha 2I$  conformational activation triggered by TPA. Thus, inside-out activation of  $\alpha 2\beta 1$  is synergistically regulated by these two activation mechanisms.

Collagen was shown to induce the  $\alpha 2\beta 1$  clustering and to initiate the specific outside-in signaling through p38. In contrast to inside-out signaling,  $\alpha 2_{E336A}\beta 1$  could not initiate fast and transient p38 activation in response to collagen induced clustering. Thus, the results indicate that concomitant receptor clustering and conformational changes in integrins are needed for integrin outside-in signaling.