

1. Introduction

1.1 Interdependence between adhesion and proliferation: The role of integrins in anchorage-dependent growth of non-lymphoid cells.

Multicellular organisms have to ensure that their cells replicate only when cell multiplication contributes to the benefit of the whole organism. Proliferation is therefore tightly regulated and normally repressed unless positive signals enable cells to replicate. Important factors determining whether a cell proliferates or not are believed to be the differentiation state of a cell, the presence of appropriate growth factors and the adhesive interactions between a cell and the surrounding micro-environment. Intercellular contacts in monolayer-forming tissues for example keep cells in a non-proliferative state (contact inhibition). Further, the proliferation of most constitutively adherent cell types not only depends on the presence of tissue-specific growth factors but as well on adhesion (anchorage-dependence): Only cells which adhere to proteins of the extra-cellular matrix, a meshwork of protein fibres embedded in a polysaccharide gel mostly found in connective tissues, replicate in response to growth factors. Over the past few years it has become clear that signal transduction and cell cycle events typically thought to be the consequence of growth factor action are actually regulated co-ordinately by growth factors, cell anchorage and a cytoskeleton-dependent "spread" cell shape (Assoian and Zhu, 1997). Both contact inhibition and anchorage-dependence are features of normal cell growth and their loss is a hallmark of neoplastic transformation. While contact inhibition is thought to be mostly mediated by cadherins (Hulsken et al., 1994), anchorage-dependence has been attributed to the integrin family of adhesion receptors (Ruoslahti and Reed, 1994). Integrins are heterodimeric cell surface receptors composed of one α - and one β -chain out of at least 16 different α - and 8 β -subunits. Integrins can mediate both adhesion to different matrix components and intercellular adhesion. Integrin-ligand binding results in integrin clustering, their recruitment to the so-called focal contacts and actin polymerization leading to the reorganization of the actin-based cytoskeleton. Notably, the pro-mitotic effect of integrins were shown to depend on the induction of a cytoskeleton-dependent spread cell shape; integrin-receptor engagement and clustering *per se* was found to be insufficient to drive growth-factor stimulated cells into the cell cycle (Chen et al., 1997). Actin polymerization is a highly dynamic process. Free actin monomers (G-actin) are sequestered by a number of actin-binding proteins. Actin is therefore prevented from polymerization unless activation of signalling pathways leads to its adenosine triphosphate(ATP)-dependent polymerization. Actin polymerization can start from nucleation points in the focal contacts, where polymerized actin (F-

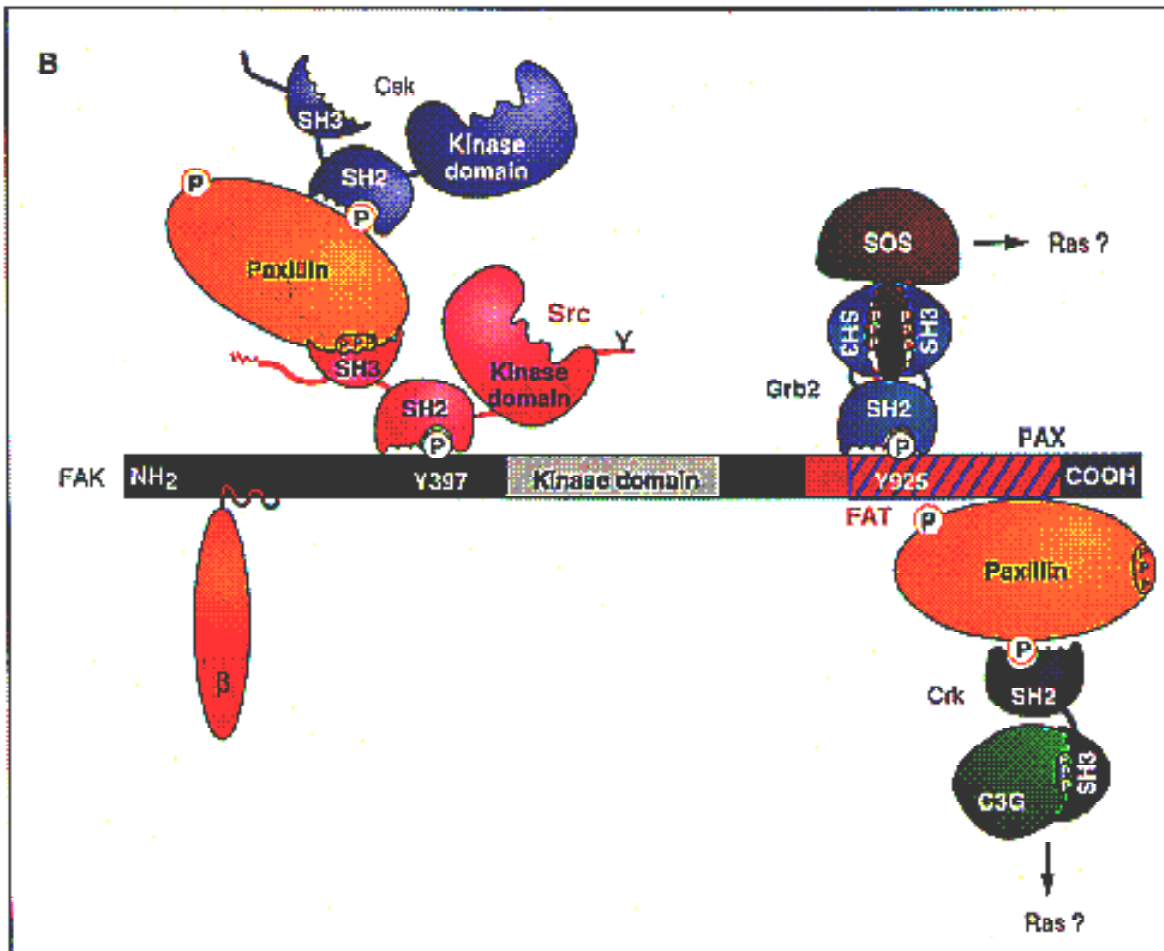
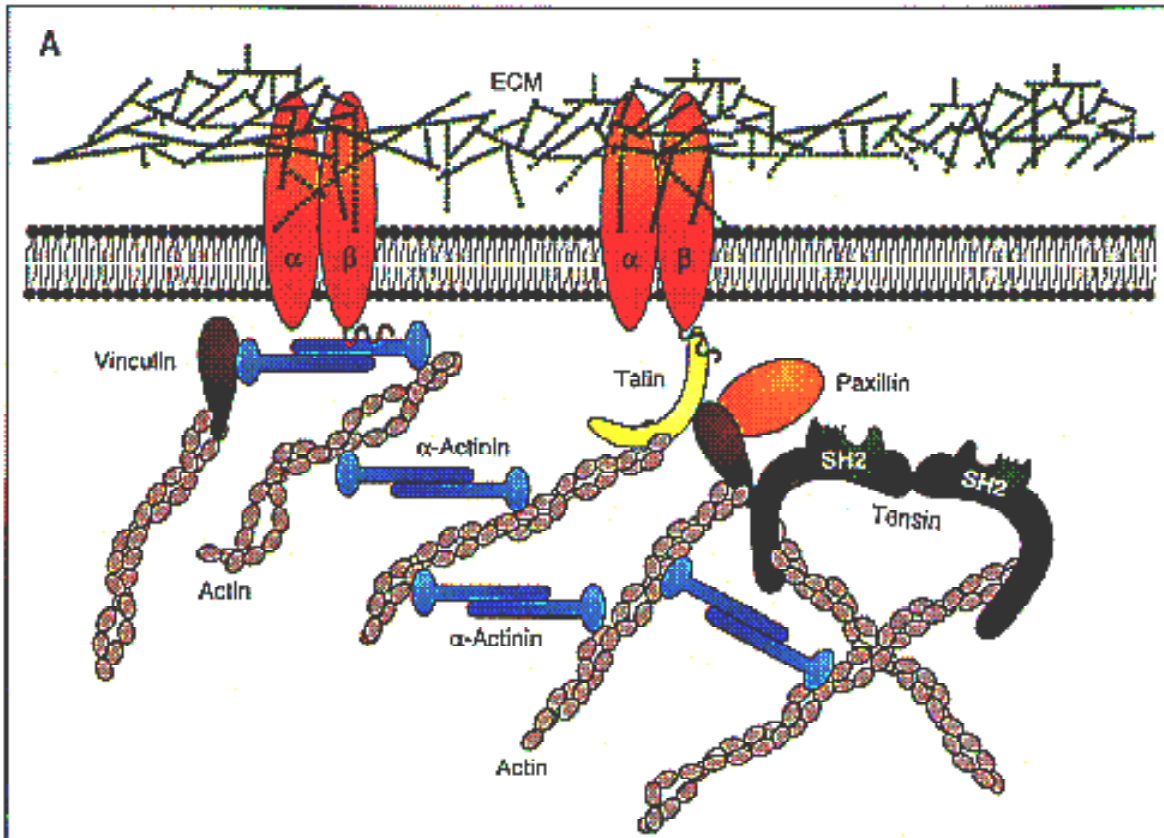


Figure 1.1a: Integrin associated proteins.

Panel A: Arrangements of cytoskeletal proteins that localize in focal adhesions and bind to integrins *in vitro*. Panel B: Signalling molecules that associate with FAK *in vivo* or *in vitro*. (E. A. Clark and J. S. Brugge: "Integrins and signal transduction pathways: the road taken" *Science* 268: 235; 1995)

Actin polymerization can start from nucleation points in the focal contacts, where polymerized actin (F-actin) is linked via actin-binding proteins to the cytoplasmic tails of integrins and therefore to the membrane. The short cytoplasmic tails of integrins do not contain intrinsic kinase or phosphatase properties like growth factor receptors. Integrin engagement and clustering is therefore believed to lead to signal transduction by the induction of large protein complexes ((Sastry and Horwitz, 1993), Figure 1.1a), which contain both cytoskeletal and catalytic signal proteins. Studies on integrin-mediated signal transduction have identified several signalling events comprising the activation of tyrosine kinases, calcium influx, the activation of several Serine/Threonine kinases like mitogen-activated protein (Map) kinases and changes in gene expression (Clark and Brugge, 1995). Some studies have proposed a central role for the focal adhesion tyrosine kinase (FAK) in integrin-mediated signal transduction, since it was reported that FAK binds to peptides mimicking the cytoplasmic $\beta 1$ -chains of integrins *in vitro* (Schaller et al., 1995).

However, other groups reported integrin- and tissue specific differences in the role of FAK in integrin-mediated signal transduction (Schaller and Parsons, 1994). Activation of FAK leads to its auto-phosphorylation and the subsequent tyrosine phosphate-dependent recruitment of other signalling mediators comprising src-tyrosine kinase family members and adapter proteins, which in turn are involved in the activation of the small guanosine triphosphate (GTP)-binding protein p21^{ras} and the Map kinase ERK (Extra-cellular signal-regulated Kinase) (Schlaepfer et al., 1994): The adapter proteins recruit exchange factors of p21^{ras} to the focal contact, resulting in the exchange of GTP to GDP bound to p21^{ras} and therefore to p21^{ras}-activation. One of the major effects of activated p21^{ras} is the recruitment of the Threonine/serine kinase Raf to the plasma membrane. This leads to Raf-activation probably dependent on phosphorylation of Raf by other kinases (Daum et al., 1994). Activated Raf phosphorylates and activates the double-specific threonine/tyrosine kinase Map kinase kinase (MKK), which in turn phosphorylates and therefore activates the ERK Map kinase. However, it has recently been proposed that integrins can activate ERK independently of p21^{ras} (Chen et al., 1996; Renshaw et al., 1997). The activation of Map kinases results in their nuclear translocation and the phosphorylation of transcription factors leading to the transcriptional activation of immediate early genes like the proto-oncogenes *c-fos* and *c-jun* ((Karin, 1996), Figure 1.1b). Map kinases are therefore believed to link signal transduction to gene expression. In the last few years, additional Map kinase cascades have been

identified (Figure 1.1.b), which are thought to be independently regulated and to contribute to growth control. While ERK is a predominantly growth factor activated kinase, the JNK (Jun-NH₂-terminal kinase) and the p38 Map kinases are thought to be activated primarily by stress like osmotic shock, inflammatory cytokines and protein translation inhibitors (Cano and Mahadevan, 1995).

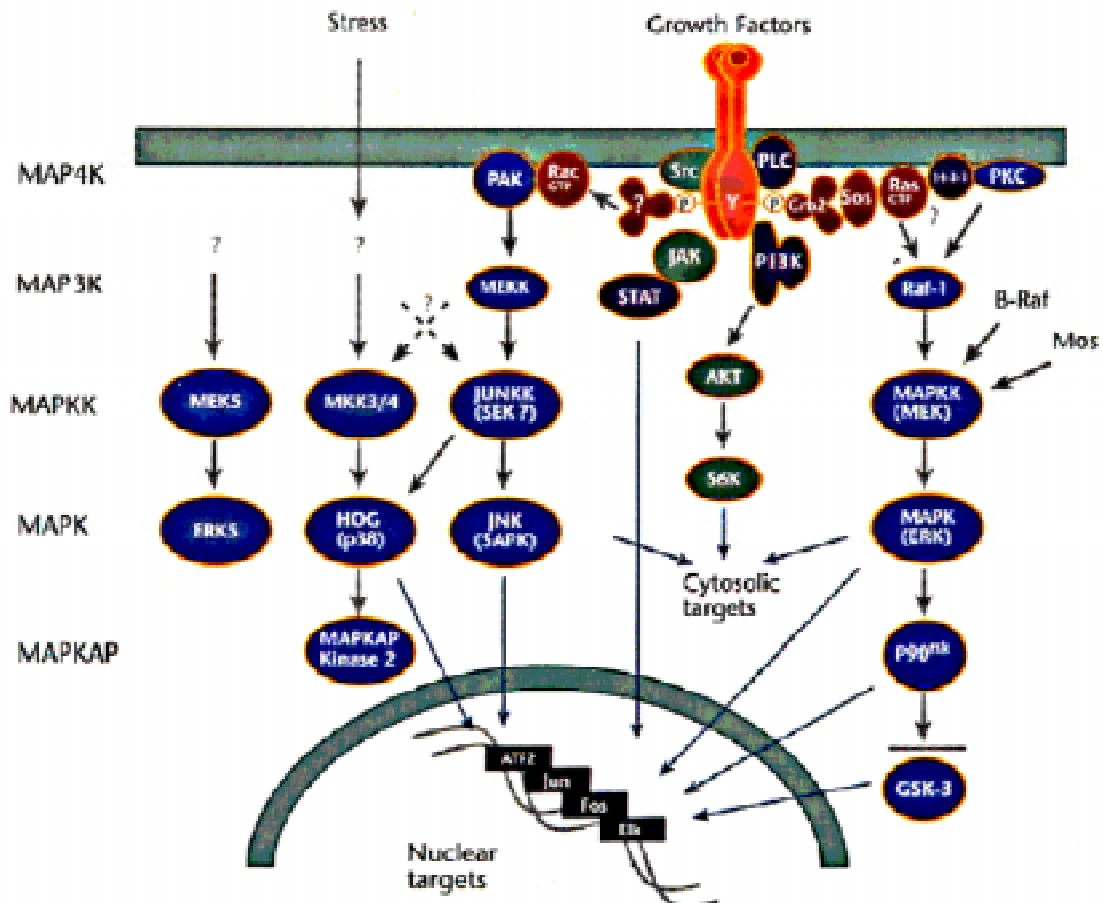


Figure 1.1b: Independent regulation of Map kinase cascades. Shown are the currently known activation cascades of Map kinases and their nuclear targets. (Sigma ImmunoNotes, No. 14, 1996).

Adhesion-dependent activation of ERK (Chen et al., 1996; Morino et al., 1995; Zhu and Assoian, 1995) and JNK (Miyamoto et al., 1995) and synergistic activation of ERK by growth factors and adhesion to extra-cellular matrix were reported (Miyamoto et al., 1996; Renshaw et al., 1997). Both anchorage-dependent cell cycle progression and the integrin-mediated activation of Map kinases was shown to require a spread cell shape, synergistic Map kinase activation was therefore proposed to link integrin-dependent signal transduction to anchorage-dependent growth (Zhu and Assoian, 1995). Mitogenic signals resulting in altered gene expression ultimately lead to cell cycle entry and/or progression; the expression of the cell cycle regulator cyclin D1 for example has been reported to depend on ERK activation (Lavoie et al., 1996).

The cell cycle is traditionally divided in several distinct phases: the M(mitotic)-phase where cells divide, and the interphase. The latter is further subdivided in the S(synthesis)-phase, where DNA replication takes place and the G(gap)-phases 1 and 2, which precede and follow DNA replication, respectively. Growth-arrested cells can leave the cell cycle and enter a resting state, called quiescence or G₀. The progression through the various phases of the cell cycle is controlled at defined stages, the so-called check points, mostly by the activation of the cyclin-dependent kinases (CDKs). The CDKs are tightly regulated by phosphorylation and complexed positive and/or negative regulators (Figure 1.1c), the cyclins and the CDK-inhibitors. The activation of the G₁ CDKs leads to the inactivating phosphorylation of the retinoblastoma tumor suppresser protein (pRb), which is required for the transition through the G₁ check point, the so-called restriction point of the cell cycle. The hyperphosphorylation of pRb results in the dissociation of the complex between pRb and transcription factors, resulting in the transcriptional activation of S phase regulators (Weinberg, 1995).

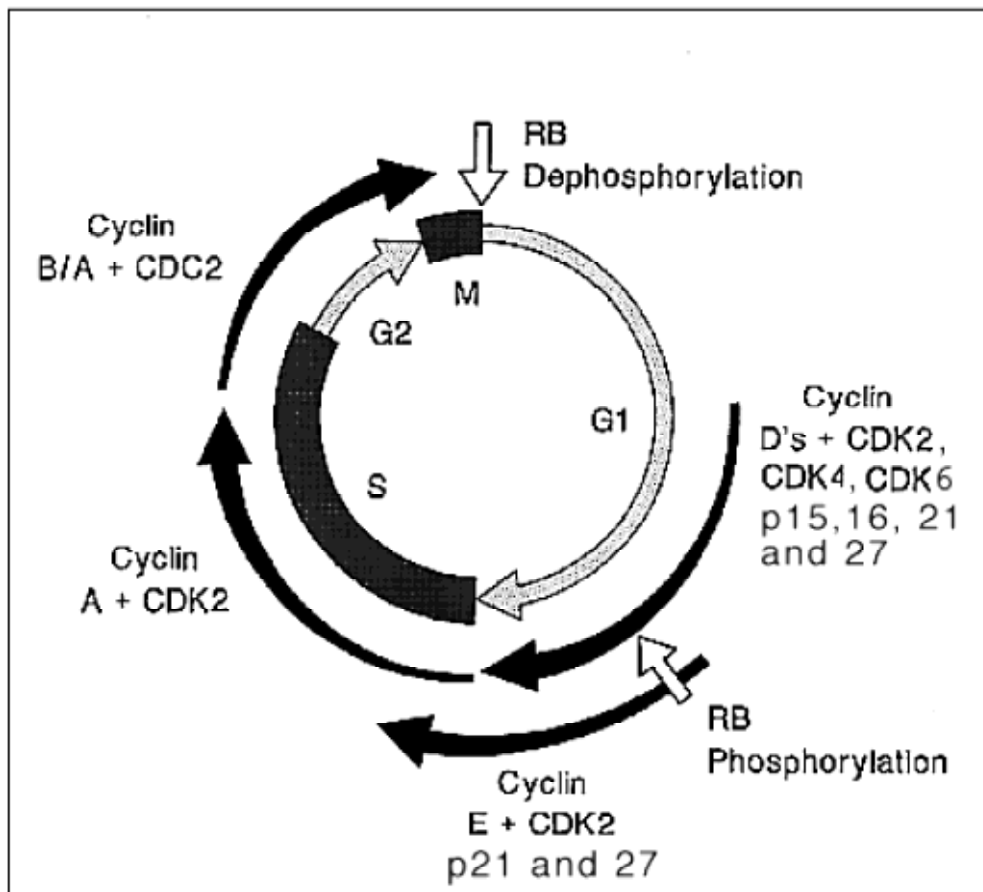


Figure 1.1c: A tentative assignment of CDK/cyclin complexes and their inhibitors to particular cell cycle transitions. The role of CDK3, 5, cyclin C, F and G and the CDK7/cyclin H complex is less clear and therefore not shown. (adapted from C. J. Sherr: "Mammalian G₁ Cyclins" *Cell* 73: 1060 1993)

Growth factors and anchorage are thought to regulate both cell cycle entry from G0 and the passage through the restriction point (Assoian, 1997; Hansen et al., 1994). Concordant with this, the expression of cyclins (D1) and the activation of the CDK/cyclin complexes due to the down-regulation of CDK inhibitors leading to pRb-inactivation and cell cycle progression are critically affected by both growth factor and anchorage ((Assoian, 1997; Bohmer et al., 1996; Fang et al., 1996; Guadagno et al., 1993; Zhu et al., 1996), Figure 1.1d).

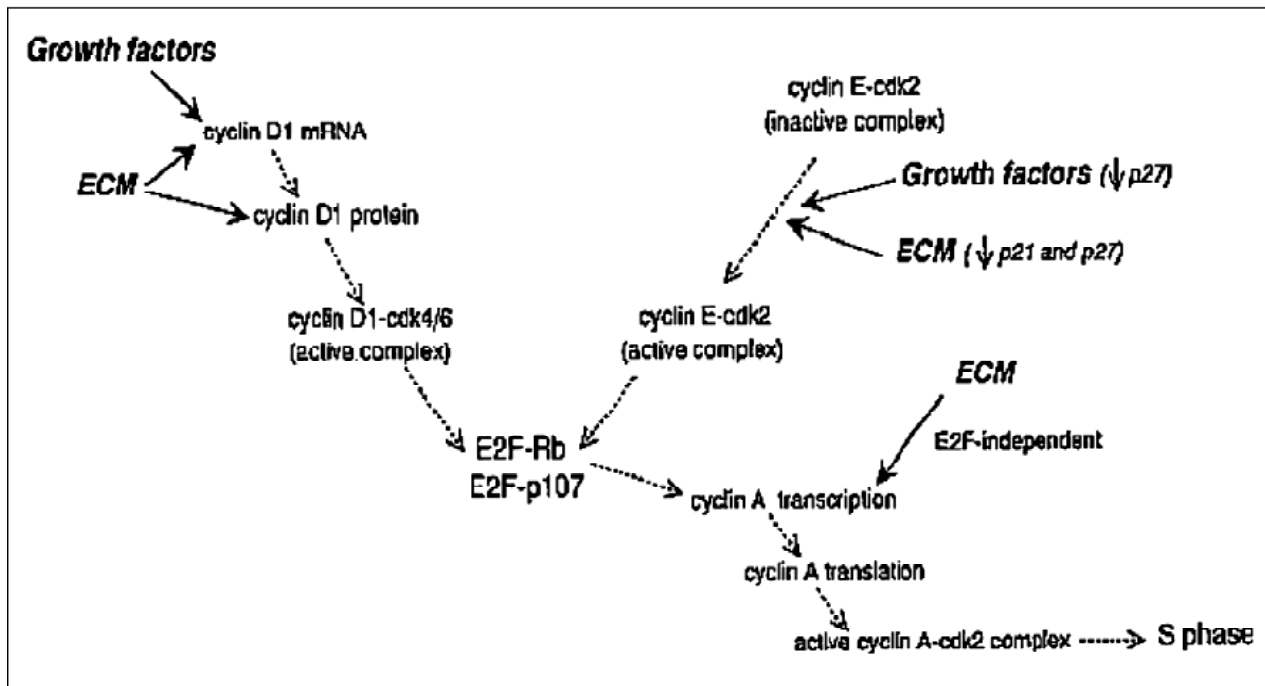


Figure 1.1d: Co-ordinated control of G1 phase cell cycle progression by growth factors and the extra-cellular matrix (ECM). A working model showing the major sites of growth factor and ECM action (solid arrows) during cell cycle progression from G0 to S phase. Regulatory phosphorylations and the roles of the INK4 proteins are not shown. (Assoian: "Anchorage-dependent cell cycle progression" *The Journal of Cell Biology* 136: 3, 1997)

In summary, the adhesion-dependence of growth factor-induced proliferation has been attributed to the capacity of integrins to mediate cytoskeletal rearrangement and activate signal transduction pathways leading to altered gene expression and cell cycle progression. Since growth factor receptor- and integrin-activated events are largely overlapping (Clark and Brugge, 1995), they have been proposed to act synergistically in promoting cell proliferation, but whether they lie on independent pathways and how these signals converge is presently unclear (Assoian and Zhu, 1997).

1.2 T lymphocyte activation

T lymphocytes are a class of white blood cells which are crucial for the antigen-directed, specific immune response of the organism against invading foreign organisms or agents. They mature from a pluripotent hemopoietic stem cell in the thymus. There only those cells survive which express a T cell antigen receptor (TCR) that binds with moderate affinity to a specific foreign peptide associated with a major histocompatibility complex (MHC-restriction). The mature T cells express different TCR-coreceptors and can therefore be subdivided in CD4(cluster of differentiation 4)-positive and CD8-positive cells, which exert typically immunoregulative and cytotoxic functions, respectively. T cells are most of the time in a non-adherent state and circulate both in the blood and the lymphatic system. Adhesion of T cells to other cells is controlled by an array of adhesion molecules which bind to their respective ligands of the opposing cell. The main classes of adhesion molecules involved in T cell adhesion are the selectins, which bind with rather low affinity to sugar groups, members of the immunoglobulin superfamily (the TCR, CD3, CD4, CD8, CD28, the ICAMs and CD2 all belong to this superfamily) and the integrins, which mediate both adhesion to other cells and binding to the extracellular matrix. Normal T cells are quiescent until they encounter an antigen presenting cell (APC) which presents their specific antigen bound to a MHC complex. The intercellular contact of an APC and a T cell can result in T cell activation, subsequent proliferation, clonal expansion, execution of the respective effector function and maturation into a state called memory or experienced T cell. Memory T cells are characterized by less stringent activation requirements as compared to T cells, which had never encountered their antigen before (virgin or naive T cells). Therefore, in spite of the fact that T cells isolated from blood are all quiescent, they are heterogeneous in their activation requirements since they contain cells expressing either the CD4- or the CD8-coreceptor and which are either of the memory or of the naive cell type (Damle et al., 1992).

One of the first measurable events after antigenic stimulation of the T cell is tyrosine phosphorylation. This comprises the phosphorylation of the ITAMs (Immunoreceptor Tyrosine-based Activation Motifs) in the CD3-receptor complex, which is stably associated with the TCR and mediates TCR-dependent signal transduction (Perlmutter et al., 1993). Various different tyrosine kinases are expressed in T cells. The src-family kinases p56^{lck} and p59^{fyn} and the syk-family kinases p72^{syk} and ZAP-70 (zeta-chain associated protein kinase of 70kD) are thought to be crucial for the onset of tyrosine phosphorylation, since they are found to be complexed to the TCR/CD3 complex and/or to the coreceptors CD4/CD8. TCR-mediated tyrosine phosphorylation of Phospholipase (PLC)- γ leads to its activation and subsequent production of diacylglycerol and inositol phosphates. This results in protein kinase C (PKC) activation and calcium mobilization, respectively. Activators of PKC (phorbol esters) and calcium mobilization (calcium ionophores) are sufficient to induce proliferation. This illustrates the

importance of PLC γ activation for T cell activation even if it is now accepted that phorbol esters activate as well p21^{ras} in T cells. The phosphorylation of the ITAMs is thought to have a similar role in TCR signalling as the proposed role of integrin-dependent FAK-autophosphorylation, since it leads to the recruitment of several additional signalling molecules (Zenner et al., 1995). The importance of tyrosine phosphorylation for T cell activation is further underlined by the fact that different tyrosine kinase inhibitors completely prevent T cell activation. The p21^{ras}-pathway is probably activated by recruiting adapter proteins to the tyrosine phosphorylated ITAMs. They bind in turn the p21^{ras}-GDP/GTP-exchange factor SOS (son of sevenless). As described in 1.1, GTP-loaded p21^{ras} activates the ERK-cascade ((Pastor et al., 1995), Figure 1.2).

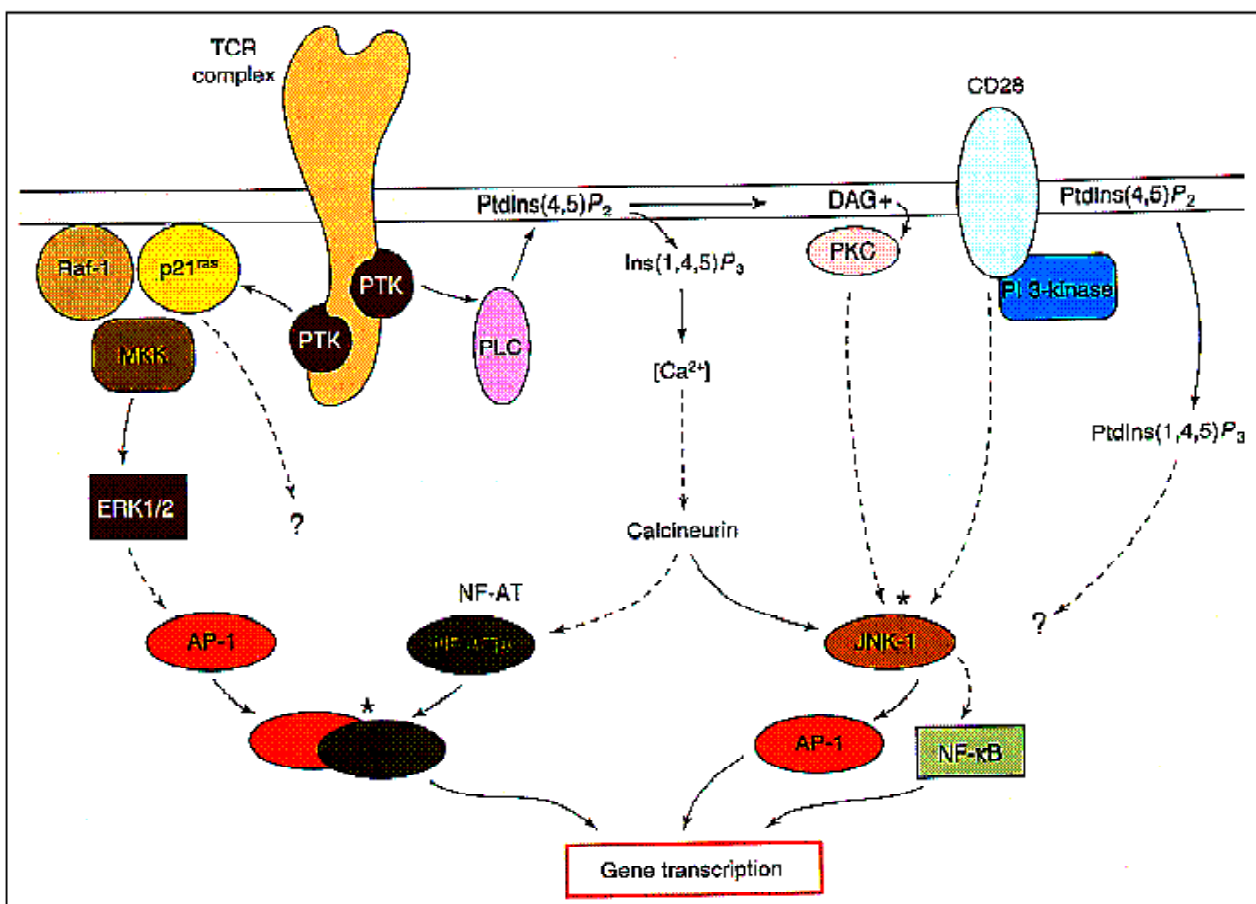


Figure 1.2: Signalling pathways that control T-cell activation. (M. I: Pastor et al.: "The regulation of p21^{ras} during T cell activation and growth" *Immunology Today* 16 (3): 163, 1995)

TCR-mediated tyrosine kinase activation leads further to the rapid internalization and degradation of the antigen receptor complex, probably representing a negative feed-back mechanism to prevent excessive signalling (Valitutti et al., 1997). T cell activation leads to the co-ordinated expression of different sets of genes (Ullman et al., 1990). It is not entirely clear how the activation of signal transduction pathways results in gene expression and

subsequently proliferation. The nuclear translocation of the transcription factors "nuclear factor kappa light-chain enhancer binding" (NF- κ B) and "nuclear factor of activated T cells" (NF-AT) and of the Map kinases are currently thought to link signal transduction to gene expression ((Karin, 1996), Figure 1.1b and 1.2). The first wave of transcriptional activation is independent of protein synthesis and begins soon after TCR stimulation. These immediate early genes encode in part transcription factors like the activator protein-1 (AP-1) components *c-jun* and *c-fos*. The newly synthesized transcription factors in turn are thought to activate a second set of genes which comprise Interleukin-2 (IL-2), the α -chain of the IL-2 receptor (CD25) and G1 cell cycle regulators.

As described in 1.1 for anchorage-dependent cell growth, the T lymphocyte restriction point is regulated through the inactivation of the tumour suppresser protein pRb by G1 CDKs: pRb is first phosphorylated in mid G1, approximately 12 hours post-stimulation, coinciding with the theoretical restriction point (DeCaprio et al., 1992). Quiescent T cells express only low levels of the pRb kinases CDK2, 4 and 6 and low or not detectable amounts of cyclins. Upon activation, cyclin D2 and CDK4 and 6 are rapidly upregulated after 4-6 hours (Lucas et al., 1995; Modiano et al., 1994), followed by cyclin D3 at 8-12 hours. In contrast, CDK2 and 5, and cyclin A and E accumulate at later time points (20-32 hours (Ajchenbaum et al., 1993)). Cyclin D1 is not expressed at all. Crucial events required for cell cycle progression of T cells are further the expression of the T cell growth factor IL-2 and of the IL-2 high affinity receptor, CD25. The expression of IL-2 is regulated both at the transcriptional and the post-transcriptional level and is inhibited by the immunosuppressant cyclosporin A (CsA). IL-2 gene transcription reflects the convergence of multiple signal transduction pathways on expression, nuclear translocation and/or post-translational modifications of different transcription factors. The identified regulatory elements in the IL-2 promoter contain consensus binding motifs for members of the NF- κ B/Rel, AP-1, NF-AT and Octamer(OCT)-family of transcription factors, whose co-operative binding is required for efficient IL-2 gene transcription (Jain et al., 1995). While AP-1-dependent gene expression is regulated by the expression levels of the AP-1 family members, their differential dimerization and phosphorylations (Angel and Karin, 1991), both NF-AT and NF- κ B-dependent transcription require further the nuclear translocation of the normally cytoplasmatic transcription factors. The nuclear translocation of NF-AT (nuclear factor of activated T cells) is induced by the dephosphorylation of NF-AT by the calcium-dependent phosphatase calcineurin, which is inhibited by CsA. In the nucleus NF-AT can heterotrimerize with an AP-1-dimer and binds to at least two different sites in the IL-2 promoter; NF-AT consensus binding sites are further nearly exclusively found in the promoter of other cytokines (Jain et al., 1995). The two most important isoforms of NF-AT involved in IL-2 transcription are NF-ATc and NF-ATp, which accumulate with different kinetics in the nucleus of activated T cells (Jain et al., 1995). In resting T cells the p65(RelA)/p50-

form of NF- κ B is cytosolic, complexed to I- κ B (Inhibitor of NF- κ B) proteins which prevent the nuclear translocation of the complex. Upon stimulation, I- κ B proteins become phosphorylated, ubiquitinated and subsequently degraded by the proteasome, leading to the nuclear translocation of RelA/p50-NF- κ B. In the nucleus RelA/p50 binds to its consensus sequences and activates transcription (Finco and Baldwin, 1995). The c-Rel/p50 heterodimer appears to be regulated similarly as RelA/p50, but in contrast to RelA/p50 it appears only after several hours of T cell stimulation in the nucleus. The p50/p50 homodimer, which is as well inducible upon stimulation, appears to repress transcription (Jain et al., 1995). NF- κ B family members can bind as well to the CD28 response element (CD28RE) of the IL-2 promoter (Lai et al., 1995). CsA inhibits the degradation of I κ B and thus the nuclear accumulation of both RelA/p50 and c-Rel/p50. The nuclear translocation of NF-AT is reversible (Timmerman et al., 1996) and the nuclear location of both NF-AT and NF- κ B is believed to require a continuous stimulation (Finco and Baldwin, 1995). In contrast, both Octamer and AP-1 family members appear to be constitutively located in the nucleus. The expression of IL-2 is further regulated by messenger-RNA stabilization: multiple AUUUA-motifs in the 3'-untranslated region of the IL-2 m-RNA are bound by regulatory proteins, which regulate the degradation rate of the m-RNA (Henics et al., 1994).

The expression of the IL-2 receptor α -chain, CD25, is positively regulated by IL-2 and leads to a high affinity of the already expressed low affinity IL-2 β/γ -receptor (Ullman et al., 1990). Resting T cells do not respond to IL-2, the "competence" to proliferate in response to the mitogen is acquired upon activation. The binding of IL-2 to its receptor is believed to be crucial for the *in vitro* proliferation, even if IL-2 may be substituted by alternative cytokines *in vivo* (Kundig et al., 1993). The mechanism by which IL-2 mediates the passage through the restriction point is not entirely clear: IL-2 has been found to mediate pRb-hyperphosphorylation in T cells stimulated with mitogenic lectins (Evans et al., 1992), and to be required for the induction of both D-type cyclins (Turner, 1993). Conversely, another report proposed a necessary role for IL-2 for CDK2, but not for the initial CDK4/cyclin D2 upregulation and pRb-directed kinase activity (Modiano et al., 1994). IL-2 has further been proposed to induce CDK2-activation and S phase entry by downregulating the pleiotropic CDK inhibitor p27^{kip1} (Firpo et al., 1994; Nourse et al., 1994) Concordant with this, T cells from p27 knock-out mice showed enhanced IL-2 responsiveness *in vitro*. (Fero et al., 1996). The role of the G1 CDK-specific inhibitors, the INK4 proteins (INHibitor of CDK4) was not investigated yet; p16^{INK4a} is not expressed in primary T cells (Tam et al., 1994).

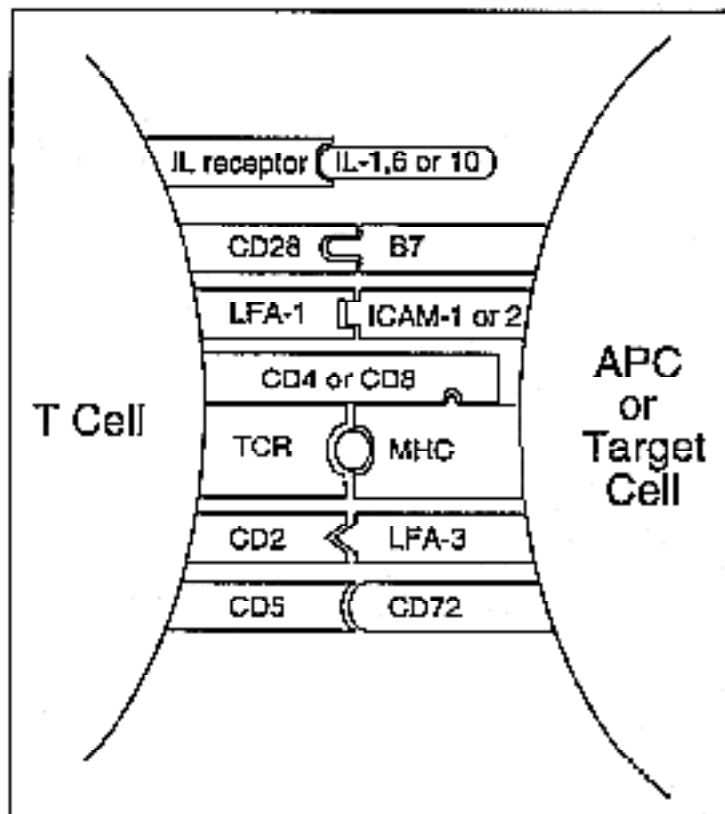
In summary, the triggering of the T cell antigen-receptor initiates a complex network of signal transduction events comprising the early activation of tyrosine kinases leading to Map kinase activation and immediate early gene expression. T cell activation results in cell cycle entry and altered expression

of genes such as G1 cyclins and IL-2 which in turn mediate pRb-inactivation, the passage through the restriction point and cell cycle progression.

1.3 The role of integrins and other costimulatory receptors in T cell activation

The interaction between the TCR and the peptide-loaded MHC complex alone appears to be normally insufficient for full T cell activation. Antigen-presenting cells therefore express additional ligands which stimulate the so-called costimulatory receptors of the T cell. Co-stimulation of TCR-dependent proliferation has been reported for a number of receptors, including CD2, CD28 and the integrins $\alpha 4/\beta 1$ (very late antigen 4, VLA-4) and $\alpha L/\beta 2$ (leukocyte function antigen 1, LFA-1) ((Croft and Dubey, 1997), Figure 1.3). Therefore a two signal model was proposed: The ligation of the TCR mediates signal 1, which is necessary but not sufficient for full T cell activation. The stimulation of costimulatory receptors induces a TCR-independent signal 2, which in synergism with signal 1 leads to full T cell activation.

Figure 1.3: Molecular interactions that occur during antigen recognition at the interface of a T cell and an antigen-presenting cell (APC) or target cell. (J. D Fraser et al.: "Signal transduction events leading to T cell lymphokine gene expression," Immunology Today 14 (7): 358, 1993)



Many studies suggest that this second signal is predominantly delivered by the interaction of CD28 with its ligands B7-1 or B7-2 (Lenschow et al., 1996; Rudd, 1996). Hallmarks of CD28 costimulation are the prevention of functional unresponsiveness ("anergy") and the induction of CsA-resistant IL-2 production. Tyrosine-phosphorylated CD28 was shown to associate with the Phosphatidylinositol (PI) 3-kinase leading to its activation. Since PI 3-kinase activation is the major link in the control of DNA synthesis in platelet-derived growth factor (PDGF)-stimulated cells, CD28-mediated PI 3-kinase activation was proposed to mediate the costimulatory effect of CD28 (Rudd et al., 1994). Furthermore, JNK was shown to be involved in CD28-dependent IL-2 production (Su et al., 1994) and a CD28 response element (CD28RE) was identified in the IL-2 promoter (Jain et al., 1995). However, most studies of T cell signal transduction and IL-2 transcription were performed in immortalized cell lines, where signal transduction is uncoupled from cell cycle progression. Important features of growth control of normal T cells may therefore be altered in T cell lines. For example, it was reported that primary T cells in contrast to T cell lines require CD28-mediated costimulation for AP-1-dependent transcriptional activation (Rincon and Flavell, 1994), which was CsA-sensitive and due to post-translational modifications. Furthermore, primary T cells from CD28 knock-out mice showed only limited defects in their proliferative response to antigenic stimulation (Green et al., 1994). This confirms earlier reports which suggested that multiple costimulatory interactions are important for the activation of primary T cells (Damle et al., 1992; Dubey et al., 1995; Semnani et al., 1994). A modified version of the two signal model has recently been proposed, suggesting that the major function of diverse accessory molecules during the initial phase of T cell activation is to augment the ability to signal through the TCR, and that the primary role of later costimulatory signals is to allow IL-2 secretion and growth (Croft and Dubey, 1997).

The β 2-integrin LFA-1 is expressed on all leukocytes. It mediates intercellular adhesion, since it binds to the plasma membrane receptors intercellular adhesion molecule (ICAM)-1, 2 and 3, which are expressed both on leukocytes and on non-hematopoietic cells. The functions of LFA-1 critically depend on transient cell-cell adhesion. The proposed roles for LFA-1 in T cell physiology are multiple and include recirculation processes in the lymphatic network, migration through endothelial lining of blood vessels, cytotoxic effector function and, as mentioned, the costimulation of the antigen-specific activation process. T cell adhesion is a tightly regulated and highly dynamic process: In contrast to integrins of adherent cell types, leukocyte integrins have to be activated by inside-out signalling in order to bind to their ligands. Aggregation of the TCR for example triggers inside-out signalling in T cells. This leads to an avidity shift of adhesion receptors such as CD2 or integrins for their ligands and subsequently to a conversion of the cell to an adherent state (Ginsberg et al., 1992). Since ligand binding of

leukocyte integrins is induced by the binding of some divalent cations or monoclonal antibodies to the extra-cellular part of the receptors, the avidity shift has been attributed to a conformational change, but the cytoskeleton-dependent receptor clustering seems to be involved as well in this process (Stewart et al., 1996). Concordant with this, the stimulation of the TCR with immobilized antibodies was shown to induce the recruitment of integrins to the actin-based cytoskeleton, paralleled by cytoskeletal rearrangement and spreading (Pardi et al., 1992). The induction of spreading was found to be partially impaired in T cells derived from patients affected by LAD (Leukocyte Adhesion Deficiency due to reduced or absent expression of β 2-integrins), indicating an important role for LFA-1 in TCR-triggered cytoskeletal rearrangement.

The existing evidence regarding the role of the cytoskeleton and LFA-1 in T cell activation is rather controversial: Several independent reports have documented the costimulatory role of LFA-1 in T cell proliferation (Damle et al., 1992; Dubey et al., 1995; Hernandez-Caselles et al., 1993; Semnani et al., 1994) Some groups suggested that the costimulatory effect of leukocyte integrins and the cytoskeleton on proliferation is not due to integrin-mediated mitogenic signalling but that it is a consequence of adhesion strengthening: Ligand-binding of LFA-1 and cytoskeletal integrity would only stabilize the adhesive contact needed for the TCR to engage its cognate ligand on the antigen-presenting cell (Bachmann et al., 1997; Berg and Ostergaard, 1995; Valitutti et al., 1995). It is indeed very likely that adhesion molecules like LFA-1 are required for a stable intercellular contact: The interaction of the TCRs and the peptide-loaded MHC-complexes is of very low avidity; only very few peptide-MHC complexes are recognized by the monospecific TCRs and the single interaction is characterized by a low affinity and a high off-rate (Corr et al., 1994). However, if adhesion strengthening was the only function of LFA-1 in T cell activation, LFA-1 should not act as a signal transducer independently of TCR triggering. In contrast, several groups reported integrin-mediated signal transduction events in lymphocytes (Kanner, 1996; Kanner et al., 1993; Maguire et al., 1995; Pardi et al., 1989; Petruzzelli et al., 1996) and synergism with TCR-mediated signals resulting in prolonged PLC γ activation and enhanced DNA-binding of transcription factors (Udagwa et al., 1996; Van Seventer et al., 1992). Furthermore, other groups showed selected defects of the *in vitro* proliferative response to antigenic stimulation of T cells derived from LFA-1 knock-out mice (Schmits et al., 1996; Shier et al., 1996), confirming results obtained with LFA-1 deficient T lymphocytes from LAD patients (Corbi, 1996). However, the clear-cut dissection of LFA-1-mediated signal transduction events from adhesion strengthening-, and therefore possibly TCR-dependent effects has proved to be difficult (Udagwa et al., 1996; Van Seventer et al., 1992)

1.4 Aims of this study

Given the dependence of growth-factor induced normal cell growth on adhesion and the emerging pivotal role of integrins and the actin-based cytoskeleton in this process, we wanted to better characterize the co-stimulatory role of the α L/ β 2 integrin LFA-1 in the proliferative response of normal T lymphocytes to antigenic stimulation: We wondered if the cell-cell adhesion could be of similar importance for T cell proliferation as the cell-matrix adhesion for the replication of constitutively adherent cell types. Since LFA-1 has been proposed to be involved in cytoskeletal rearrangement induced by the TCR (Pardi et al., 1992; Stewart et al., 1996) and given the importance of a cytoskeleton-dependent spread cell shape in anchorage-dependent growth, we wanted to elucidate the role of the actin-based cytoskeleton in T cell proliferation. Clearly we had to test the current hypothesis if the observed LFA-1-enhanced proliferation could be a consequence of adhesion strengthening, or if alternatively, LFA-1-triggered, possibly TCR-independent signal transduction events could be responsible for the phenomenon. Further, the investigation of the relative contribution of the “professional costimulatory receptor”, CD28 and the “professional adhesion receptor”, LFA-1 to TCR-dependent proliferation was thought to contribute to answer the underlying question of this study: Do T cells show adhesion requirements for proliferation similar to anchorage-dependent cells? or, in other words: How differ non-adherent cells exemplified by primary T cells in the adhesion-dependence for cell growth as compared to the well-studied adherent cell types?