Interdependence of cdk2 Activation and Interleukin-2Ra Accumulation in T Cells*

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We have shown previously that serum promotes T cell proliferation by acting with T cell receptor (TCR) agonists to efficiently down-regulate p27Kip1 and activate cdk2-containing complexes. In the studies described here, the effect of serum on the expression of the α subunit of the interleukin-2 receptor (IL-2Ra) was examined. We found that serum was required for maximal and sustained IL-2Ra protein expression and consequent IL-2 signaling in TCR-activated splenocytes. Serum had no effect on IL-2Ra mRNA levels and thus modulates IL-2Ra expression post-transcriptionally. Unlike wild-type splenocytes, splenocytes exhibiting serum-independent cdk2 activation due to loss of p27Kip1 efficiently expressed IL-2Ra in serum-deficient medium. Conversely, serum did not promote IL-2Ra accumulation in conditions in which cdk2 activity was blocked. These findings demonstrate that cdk2 activation is necessary and sufficient for IL-2Ra accumulation in TCR-stimulated splenocytes. On the other hand, IL-2 signaling was required (at least in part) for cdk2 activation in these cells. Thus, cdk2 activation, IL-2Ra expression, and IL-2 signaling are interdependent events, and we suggest that this feed-forward regulatory loop plays a key role in T cell mitogenesis.

Engagement of T cell receptors (TCRs)$¹ initiates a complex program of events that leads to the proliferation and differentiation of mature resting T cells (1). A key player in this program is interleukin-2 (IL-2), a lymphokine first identified in supernatants of antigen-primed T cells (2–4). The receptor for IL-2 (IL-2R) is noncatalytic and consists of three subunits termed IL-2Ra, IL-2Rβ, and IL-2Rγ (also known as γc) (5). IL-2Rβ and IL-2Rγ are also components of other lymphokine receptors, whereas IL-2Ra is unique to the IL-2R and thus is responsible for substrate specificity. IL-2Rγ is also required for high affinity (and presumably biologically relevant) IL-2 binding. In vitro, IL-2Rs lacking IL-2Ra are functional in human T cells (albeit at a lower affinity than trimeric receptors) but not in mouse T cells (6–10). On the other hand, IL-2Ra has an extremely short cytoplasmic domain and does not participate in intracellular signaling per se. Instead, signaling is mediated by IL-2Rβ and IL-2Rγ, which interact with a variety of cytoplasmic effector proteins (11). These include the Janus kinases (JAKs), Jak1 and Jak3, which associate constitutively with IL-2Rβ and IL-2Rγ, respectively. When activated by ligand-induced receptor oligomerization, JAKs phosphorylate IL-2Rβ and IL-2Rγ on specific tyrosine residues to create docking sites for other effectors. Additional JAK substrates include the JAKs themselves and members of the STAT (signal transducer and activator of transcription) family of transcriptional regulators.

Of the three IL-2R subunits, IL-2Ra exhibits the most variable expression. IL-2Ra is not present in resting T cells and is transcriptionally up-regulated by TCR agonists (12). Such agonists include cognate antigen, anti-CD3, and concanavalin A (ConA). IL-2Rβ and IL-2Rγ, on the other hand, are expressed constitutively, and TCR-induced changes in their expression are less dramatic (5). TCR activation increases IL-2 production (13), and IL-2 signaling further up-regulates IL-2Ra expression in a STAT-dependent manner (14–16). As a further prelude to IL-2 actions, TCR stimulation induces a variety of metabolic responses that allow quiescent T cells to exit G0 (11). IL-2 then promotes continued G0/G1 traverse and the initiation of DNA synthesis. T cell proliferation is also influenced by co-stimulatory signals delivered by CD28 (17) and by mitogens contained in serum, which is indispensable for T cell propagation in vitro (18). The capacity of co-stimulatory signals to up-regulate IL-2Ra transcription has been reported (19).

As in all cell types, T cell proliferation is governed by the ordered activation of cyclin-dependent kinases (CDKs) (20). CDK activity is controlled by cyclins, which are positive regulators, and CDK inhibitors, which repress activity. Cyclins, which are expressed periodically, combine with CDKs to form active complexes at distinct points in the cell cycle. During G1/S, for example, complexes containing the D cyclins and cdk4 or cdk6, cyclin E and cdk2, and cyclin A and cdk2 are sequentially assembled and activated. Activation of these complexes is required for G1/S traverse and S phase entry, and key substrates include the Rb family of transcriptional repressors. The CDK inhibitor family includes the Cip/Kip proteins, which inactivate complexes containing cdk2 and, according to some reports, cdk4 and cdk6 (21–23). Of these inhibitors, p27Kip1 is thought to play a particularly important role in T cell proliferation. p27Kip1 is present at high levels in resting T cells and is down-regulated in response to mitogenic stimulation (24, 25). Failure to reduce p27Kip1 levels below a critical threshold precludes cdk2 activation and arrests T cells in G0/G1. Moreover, T cells lacking p27Kip1 exhibit dysregulated cdk2 activation and proliferate in conditions that do not support the
growth of wild-type cells (Ref. 26 and accompanying article (43)). Previous studies have shown that IL-2 elicits and is required for efficient $p27^{kip1}$ down-regulation in primary T cells and T lymphoblasts (24, 25). In addition, we have found that serum acts with TCR agonists to maximally and persistently reduce $p27^{kip1}$ levels and, consequently, to activate cdk2 in naive T cells (43).

Given the pivotal role of IL-2 in T cell mitogenesis, a full understanding of the mechanisms regulating the expression of IL-2 and IL-2R is imperative. IL-2R density is a critical determinant of T cell proliferation (27), and previous studies have shown that IL-2R density is dictated by serum concentration (18). As monitored by IL-2 binding assays, serum (at 10%) significantly increased the expression of surface-localized IL-2Rs in antigen-treated human T cells (18). Building on this observation, data presented here demonstrate that serum selectively facilitates the post-transcriptional expression of IL-2Rα in primary splenic T cells exposed to TCR agonists. Serum-mediated IL-2Rα accumulation was accompanied by the induction of IL-2 signaling pathways and was dependent on cdk2 activation. Because cdk2 activity both contributed to and resulted from IL-2 signaling, we suggest that these events are interdependent. We propose a model of T cell proliferation in which serum-dependent $p27^{kip1}$ down-regulation initiates a feed-forward loop consisting of cdk2 activation, IL-2Rs accumulation, and IL-2R signaling.

**EXPERIMENTAL PROCEDURES**

*Isolation of Splenocytes and Cell Culture—*A single cell suspension of splenocytes was prepared by passage through nylon mesh, and red cells were depleted using a whole blood erythrocyte lysing kit (R&D Systems). For purification, spleen cell suspensions were loaded onto T cell-enrichment columns (R&D Systems), and T cells were isolated by high affinity negative selection as specified by the manufacturer. Splenic and purified T cells were plated at 10⁶ cells/ml and 5 × 10⁶ cells/ml, respectively, in RPMI 1640 supplemented with 50 units/ml penicillin, 2 mM l-glutamine, and 10% fetal calf serum. p27–47 fibroblasts were prepared and maintained as described previously (28). The percentage of S phase cells in a population was determined by FACS analysis of propidium iodide-stained cells as detailed previously (43).

*Protein Analysis—*Western blots were performed as described previously (43). For ELISA assays, flat bottom 96-well microtiter plates were coated overnight at 4 °C with 6 μg/ml IL-2Rα monoclonal antibody (PC61, PharMingen) and blocked with 3% bovine serum albumin for 1 h at 37 °C. Coated plates were treated consecutively as follows: serial dilutions of cell cultures (10% serum), 1 h at 37 °C; 100,000 cpm biotin-conjugated IL-2Rα monoclonal antibody (7D4, PharMingen), 1 h at 4 °C; streptavidin-biotinylated peroxidase complex (PharMingen), 1 h at 37 °C. Reactions were developed for 30 min with 100 μW/mM 3,5,5'-tetramethylbenzidine substrate (Dako) and stopped with 0.5 N H₂SO₄. Absorbance was measured at 450 nm on a Titertek ELISA reader. This protocol is a modified version of Osawa et al. (29). For analysis of cell surface marker expression, cells were incubated in phosphate-buffered saline containing 2% mouse IgG (Dako) and fluorescein isothiocyanate- or phycoerythrin-conjugated antibodies (PharMingen) for 30 min in the dark at 4 °C. Corresponding isotype-specific conjugated antibody was used for detection of nonspecific binding. Analysis was performed on a FACSscan flow cytometer with Cell Quest software (Becton Dickinson).

*mRNA Analysis—*Total mRNA was isolated using TRIzol, and Northern blots were performed as described previously (30). For RNAse protection assays, mRNA (20 μg) was hybridized overnight at 56 °C with 32P-labeled probes (10°C) corresponding to the mCR-1 probe set (PharMingen). Samples were then digested with RNase T1 and RNase A for 45 min at 30°C and proteinase K for 15 min at 37°C. Samples were extracted with phenol/chloroform, collected by sodium acetate/ethanol precipitation, denatured at 90°C for 3 min, and electrophoresed on a 5% polyacrylamide gel. Gels were dried and exposed to x-ray film.

*In Vitro Kinase Assay—*Cell extracts were incubated with antibody to cyclin E or cyclin A for 4–12 h at 4 °C and subsequently with protein Aagarose beads. Immune complexes were washed twice with lysis buffer (43) and once with histone kinase buffer (50 mM Tris (pH 7.4), 10 mM MgCl₂, 1 mM dithiothreitol). Washed complexes were incubated in 15 μl of kinase buffer containing 20 μM ATP, 0.1 μM [γ-32P]ATP and 100 μg/ml histone H1 (Roche Molecular Biochemicals) for 10 min at 37 °C. Reactions were stopped by boiling in Laemmli buffer, and proteins were separated on SDS gels. Radiolabeled proteins were visualized by autoradiography.

**RESULTS**

*Serum Modulates IL-2Rα Expression at a Post-transcriptional Level—*Although the serum dependence of IL-2R expression has been described previously (18), the receptor component(s) targeted by serum has yet to be identified. To address this issue, purified splenic T cells derived from Balb/c mice were stimulated for 30 h with mitogenic concentrations of anti-CD3 and either 10% or 0.1% serum, and the cell surface expression of IL-2Rα and IL-2Rγ was determined by FACS analysis. These serum concentrations were chosen because they allow maximal (10% serum) and minimal (0.1% serum) amounts of DNA synthesis in anti-CD3-treated T cell cultures (Fig. 1A, top panel, and accompanying article (43)). As shown in Fig. 1B, resting T cells contained little if any surface-localized IL-2Rα, and a higher percentage of cells expressed this protein when stimulated with anti-CD3 and 10% as compared with 0.1% serum (76% versus 33%, respectively). Moreover, there were significantly more receptors per cell in the population receiving 10% serum as compared with 0.1% serum (Fig. 1C). On the other hand, one-third of the cells in the quiescent population were IL-2Rγ-positive, and anti-CD3 increased this percentage ~2-fold irrespective of serum concentration (Fig. 1B). These data show that serum regulates the overall expression and/or the cell surface localization of IL-2Rα, but not of IL-2Rγ, in TCR-stimulated T cells. Levels of IL-2Rβ detected by this assay were too low to be accurately quantitated.

To ensure that T cells remained viable when stimulated in serum-deficient medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays were performed. When exposed 40 h after stimulation, T cells treated with anti-CD3 and 0.1% serum were only slightly less viable than those receiving anti-CD3 and 10% serum (Fig. 1A, bottom panel). This change in viability is not sufficient to account for the low levels of IL-2Rα expression and S phase entry in T cell populations exposed to anti-CD3 and 0.1% serum. We also found that the serum requirement for DNA synthesis and IL-2R expression could not be overridden by high levels of IL-2 or by antibody to CD28 (Fig. 1A, top panel, and data not shown). These results suggest that these signals are not rate-limiting in cells in serum-deficient medium. Although cells receiving anti-CD3 and 0.1% serum did not initiate DNA synthesis, they did exhibit increased expression of the early T cell activation markers, CD69 and CD28 (Fig. 1B). This finding is consistent with our earlier report showing that TCR-stimulated T cells partially traverse G₁/G₂ in serum-deficient medium (43).

To determine if serum concentration affected total (as well as surface-localized) levels of IL-2Rα, ELISAs and Western blots were performed on whole cell extracts. To expedite these studies, unfractionated splenocytes and purified T cells exhibited identical responses (43). As shown in Fig. 2, A and B (left panel), IL-2Rα was barely detectable in resting splenocytes and was weakly induced by ConA at 10 h regardless of serum concentration. After this time, IL-2Rα
continued to accumulate in cells co-stimulated with ConA and 10% serum, with peak expression occurring at 30–40 h. In contrast, IL-2Rα essentially disappeared from cells receiving ConA and 0.1% serum. The capacity of serum to enhance IL-2Rα expression was also evident in splenocytes stimulated with anti-CD3 (Fig. 2B, right panel). These findings indicate that serum increases the cell surface expression of IL-2Rα, at least in part, by increasing total cellular levels of IL-2Rα. On the other hand, serum did not affect the expression of IL-2Rβ or IL-2Rγ (Fig. 2, B and C).

To assess the functional consequences of serum-dependent IL-2Rα up-regulation, we assayed the activity of the IL-2 signaling intermediate, Jak3, in splenocytes treated with ConA and either 10% or 0.1% serum. To detect active Jak3, Jak3 immunoprecipitates were immunoblotted with antibody to phosphotyrosine. As presented in Fig. 3A, increases in IL-2Rα expression were paralleled by increases in Jak3 phosphorylation. Quiescent cells contained little if any IL-2Rα or phospho-
ylated Jak3. Cells receiving ConA and 0.1% serum exhibited minor increases in IL-2Rα expression and Jak3 phosphorylation, whereas both responses were substantially elevated in cells exposed to ConA and 10% serum. Serum (at 10%) also increased IL-2Rα levels and activated Jak3 when added to cells 20 h after addition of ConA and 0.1% serum. This result shows that cells incubated with ConA in serum-deficient medium remain viable and retain the capacity to initiate IL-2-dependent events when subsequently exposed to 10% serum.

To determine if serum regulated IL-2Rα mRNA expression, RNase protection assays were done on splenocytes treated with ConA and 10% versus 0.1% serum. As shown in Fig. 3B, IL-2Rα mRNA levels rose within 5 h of addition of ConA to cells and remained elevated for up to 25 h regardless of serum concentration. This finding demonstrates that maximal increases in IL-2Rα mRNA levels are not sufficient for maximal expression of IL-2Rα protein and, consequently, that serum

**FIG. 1.** Proliferation, survival, and cell surface marker expression of purified T cells as a function of serum concentration. A, resting T cells received the indicated combinations of anti-CD3 (5 µg/ml), anti-CD28 (2 µg/ml), IL-2 (1000 units/ml), and either 10% or 0.1% serum. Top panel, triplicate cultures were pulsed with 1 µCi/ml [3H]thymidine for 16 h prior to harvest at 48 h. Incorporation was determined by scintillation counting, and results are expressed as counts per minute ± the standard deviation. Bottom panel, MTT assays were done on triplicate cultures at 40 h after stimulation as specified by the manufacturer (R&D Systems). Results are expressed as absorbance at 550 nm ± the standard deviation. B and C, resting T cells were stimulated with 5 µg/ml anti-CD3 and either 10% or 0.1% serum for 30 h. The cell surface expression of CD69, CD28, IL-2Rα (CD25), and IL-2Rγ (CD132) was determined by FACS analysis using the appropriate antibodies. B, data are expressed as the percentage of positive cells. C, the histogram for IL-2Rα is shown. Background fluorescence (left peak) was determined using an isotype control antibody, and specific fluorescence (right peak) was determined using an IL-2Rα antibody.

**FIG. 2.** Effect of serum on the overall expression of IL-2Rα, IL-2Rβ, and IL-2Rγ. A, quiescent splenocytes received 2.5 µg/ml ConA and either 10% serum (squares) or 0.1% serum (circles) for the indicated times. IL-2Rα protein levels were determined by ELISA. B, resting splenocytes were treated with either 2.5 µg/ml ConA (left panel) or 5 µg/ml anti-CD3 (right panel) and the indicated amounts of serum for the indicated times. IL-2Rα and IL-2Rβ protein levels were determined by Western blotting. C, resting splenocytes were stimulated with 2.5 µg/ml ConA and either 10% or 0.1% serum for 24 h. IL-2Rα, IL-2Rβ, and IL-2Rγ levels were determined by Western blotting.
controls IL-2Rα expression at a post-transcriptional level. Similar to protein levels, mRNA levels of IL-2Rβ and IL-2Rγ were unaffected by serum concentration.

**Serum-dependent IL-2Rα Expression Requires cdk2 Activity**—The data presented above show that T cells do not appreciably express IL-2Rα, activate JAKs, or enter S phase when stimulated with medium containing ConA and anti-CD3 and 0.1% serum. On the other hand, we have found that splenocytes derived from p27kip1-null C57Bl/6 mice are capable of initiating DNA synthesis when exposed to a TCR agonist and either 10% or 0.1% serum (Fig. 4 and accompanying article (43)). This finding implies that cells lacking p27kip1 either efficiently express IL-2Rα in serum-deficient medium or no longer require IL-2 signaling for proliferation. To distinguish between these alternatives, we examined IL-2Rα expression and Jak1 and Jak3 activity in p27kip1-/- splenocytes exposed to ConA and either 10% or 0.1% serum. IL-2Rα was not detectable in quiescent p27kip1-/- splenocytes but was present at high levels in ConA-treated p27kip1-/- splenocytes regardless of serum concentration (Fig. 4). Moreover, both Jak1 and Jak3 were active in p27kip1-/- splenocytes receiving ConA and either 10% or 0.1% serum. Similar to Balb/c splenocytes, wild-type C57Bl/6 splenocytes required 10% serum for both IL-2Rα accumulation and JAK activation. These data indicate that the capacity of p27kip1-/- splenocytes to proliferate in serum-deficient medium results (at least in part) from the capacity of these cells to optimally express IL-2Rαs in a serum-independent manner.

In wild-type splenocytes, cdk2 activation requires a pronounced and persistent decrease in p27kip1 levels, and both ConA and 10% serum are needed to achieve this effect (43). In contrast, in p27kip1-null splenocytes, cyclin E-cdk2 activation is constitutive and cyclin A-cdk2 activation is serum-independent (23, 34, 43). Thus, conditions that promote cdk2 activation are the same as those that promote IL-2Rα expression, and it is possible, therefore, that cdk2 activity contributes to IL-2Rα expression. In support of this hypothesis, we found that IL-2Rα did not accumulate in Balb/c splenocytes treated with ConA and 10% serum in the presence of roscovitine, a potent and selective inhibitor of cdk2 activity (Fig. 5A and Ref. 32). Roscovitine did not inhibit the expression of cyclin E and thus does not nonspecifically block protein synthesis. Similar to naive splenocytes, roscovitine also precluded IL-2Rα expression, as well as cdk2 activity, in quiescent T lymphoblasts restimulated with ConA and 10% serum (Fig. 5B).

The necessity of cdk2 activity for IL-2Rα accumulation was further demonstrable in experiments in which IL-2Rα was transiently expressed in a fibroblast cell line (termed p27-47) that inductively expresses p27kip1 in response to isopropyl-β-D-thiogalactopyranoside (IPTG) (Fig. 5C and Ref. 28). As we reported previously, induction of p27kip1 in sparse p27-47 cells represses cdk4 and cdk2 activity but does not result in growth inhibition (28). As shown in Fig. 5C, IL-2Rα mRNA and protein were apparent in p27-47 cells transfuscated with a plasmid containing IL-2Rα cDNA but not with vector alone. Although IL-2Rα mRNA levels were approximately equal in cells treated with or without IPTG, IL-2Rα protein levels were substantially lower in IPTG-treated as compared with untreated cultures. On the other hand, levels of ectopically expressed β-galactosidase were similar in both IPTG-treated and untreated cultures, thus indicating that p27kip1 overexpression does not globally inhibit protein expression. Collectively, the data in Fig. 5 show that cdk2 activity is required for IL-2Rα expression. Our studies, therefore, establish a series of events in which serum facilitates cdk2 activation, which in turn modulates IL-2Rα expression at a post-transcriptional level.

**cdk2 Activity and IL-2 Signaling Comprise a Regulatory Loop**—Previous studies have shown that IL-2 stimulates cyclin E-cdk2 and cyclin A-cdk2 activity in activated T cells (33–35). Because these investigations place cdk2 activation downstream of IL-2R activation, the need for cdk2 activity for IL-2Rα accumulation seems paradoxical. It is possible, however, that these processes are interdependent; i.e. cdk2 activity enhances IL-2Rα expression and consequent IL-2 signaling promotes cdk2 activation. To assess the dependence of cdk2 activation on IL-2 signaling in our system, we assayed cdk2 activity in splenocytes treated with ConA and 10% serum in the presence or absence of an IL-2Rα blocking antibody. As shown in Fig. 6A, cdk2 activity was substantially lower in antibody-treated cultures, as was IL-2Rα expression. AG490, a selective inhibitor of Jak activity (36), also blocked cdk2 activation and IL-2Rα expression when presented to cells 12 h after stimulation with ConA and 10% serum (Fig. 6B). AG490 also repressed the expression of cyclin A and cdk2; we have shown previously that the expression of these proteins in T cells requires cdk2 activity (43). On the other hand, AG490 had no effect on cyclin...
growing p27–47 fibroblasts treated with or without 1 mM IPTG for 20 h was ascertained by hybridization of the membrane with blotting and Northern blotting, respectively. Equal RNA loading was assessed as in (A) H1 Btery. IL-2R monitored by green fluorescence protein expression using flow cytom-

Transfection efficiency was determined by Western blotting. cdk2 activity (H1 (A)) was measured in cyclin A immunoprecipitates by in vitro kinase assay. 

**DISCUSSION**

Engagement of the TCR results in the transcriptional up-regulation of IL-2Rα, and numerous studies have focused on the pathways and promoter elements involved in this response (5). Data presented here show that IL-2Rα expression is also regulated post-transcriptionally and that serum and cdk2 play prominent roles in this process. In splenocytes exposed to ConA and 0.1% serum, IL-2Rα was only weakly and transiently expressed. In contrast, in splenocytes receiving ConA and 10% serum, IL-2Rα expression was robust and sustained. Serum also elevated the cell surface expression of IL-2Rα in purified T cells, and IL-2Rα accumulation was accompanied by activation of the IL-2 signaling intermediates, Jak1 and Jak3. On the other hand, serum had no effect on IL-2Rα mRNA levels or on the expression of IL-2Rβ or IL-2Rγ at either the protein or message level. These findings clearly show that serum selec-

![Image](75x483 to 272x730)

**FIG. 5. Inhibition of IL-2Rα accumulation by roscovitine and p27Kip1 overexpression.** A, resting Balb/c splenocytes were treated with 2.5 μg/ml ConA and 10% serum prior to addition of either DMSO (vehicle control) or roscovitine (final concentration, 25 μM). Cells were harvested at the indicated times. IL-2Rα and cyclin E levels were determined by Western blotting. B, to prepare T lymphoblasts, splenocytes were treated with 5 μg/ml ConA and 10% serum for 48 h and 10% serum alone for an additional 48 h. Quiescent lymphoblasts then received 2.5 μg/ml Con A and 10% serum and either Me2SO (DMSO) or 25 μM roscovitine for the indicated times. IL-2Rα expression was determined by Western blotting, and cdk2 activity (A (H1)) was determined in cyclin A immune complexes by in vitro kinase assay. C, exponentially growing p27–47 fibroblasts treated with or without 1 mM IPTG for 20 h were transfected with a vector alone (pIRES2-EGFP) or vector containing IL-2Rα cDNA by LipofectAMINE (Life Technologies). Both sets of cells were cotransfected with β-galactosidase (β-gal) under control of the cytomegalovirus promoter. After transfection, cells were incubated with or without IPTG for an additional 20 h. Transfection efficiency was monitored by green fluorescence protein expression using flow cytometry. IL-2Rα protein and mRNA levels were determined by Western blotting and Northern blotting, respectively. Equal RNA loading was ascertained by hybridization of the membrane with β-actin cDNA probe. cdk2 activity (A (H1)) was assessed as in B. Data showing β-galactosidase protein levels in mock-transfected cells and β-galactosidase-transfected cells are also presented.

E levels and thus does not inhibit protein expression in general. Together, the above findings show that cdk2 activation both results from and contributes to IL-2Rα expression and that cdk2 activity, IL-2Rα expression, and IL-2 signaling comprise a regulatory loop.

Because IL-2 signaling is thought to be a cause rather than an effect of cdk2 activation in T cells (24, 25), we considered the possibility that these processes were interdependent. In support of this hypothesis, we found that abrogation of IL-2 signaling repressed both cdk2 expression and cdk2 activity in splenocytes stimulated with ConA and 10% serum. Two methods were used to inhibit IL-2 signaling: an IL-2Rα blocking antibody and the JAK inhibitor, AG490. On the basis of these data and those discussed above, we propose the following regulatory loop (Fig. 7). We suggest that this loop begins with cyclin E-cdk2 activation, which results from persistent serum-dependent p27Kip1 down-regulation. As described by others (37–39), cyclin E-cdk2 complexes, together with cyclin D-containing complexes, phosphorylate Rb, and thus allow the E2F-mediated transcription of cyclin A and the consequent formation of cyclin A-cdk2 complexes. As shown here, activation of cyclin E-cdk2 also leads to the post-transcriptional accumulation of IL-2Rα and
The subsequent induction of IL-2-mediated events, as exemplified by JAK activation. IL-2 signaling pathways, in turn, further optimize and sustain cyclin E-cdk2 and cyclin A-cdk2 activity. At this point in the cycle, it is likely that both of these activities promote the continued expression of IL-2Rα.

The capacity of serum to facilitate splenocyte proliferation by initiating the regulatory loop outlined in Fig. 7 is clearly demonstrated by our studies on p27Kip1-deficient splenocytes. In these cells, cdk2 activation, IL-2Rα expression, IL-2 signaling, and expression and activation of cyclin A-cdk2 activity. At this point, both cyclin E-cdk2 and cyclin A-cdk2 complexes promote continued IL-2Rα expression and subsequent events. Treatment of cells with roscovitine, anti-IL-2Rα, or AG490 stops the cycle at the point indicated and results in the inhibition of all events in the cycle.

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