Lienhard, G. E., Secemski, I. I., Koehler, K. A., & Lindquist, R. N. (1971) *Cold Spring Harbor Symp. Quant. Biol. 36*, 45–51.
Lindquist, R. N., Lynn, J. L., Jr., & Lienhard, G. E. (1973) *J. Am. Chem. Soc. 95*, 8762–8768.
MacDonald, T. L., & Martin, R. B. (1988) *Trends Biochem. Sci. 13*, 15–19.
MacDonald, T. L., Humphreys, W. G., & Martin, R. B. (1987) *Science 236*, 183–188.
MacNeal, R. L., & Purich, D. L. (1978) *Arch. Biochem. Biophys. 191*, 233–243.
Margolis, R. L. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1586–1590.
Martin, S. R., & Bayley, P. M. (1987) *Biophys. Chem. 27*, 67–76.
Mitchison, T., & Kirschner, M. (1984) *Nature 312*, 237–242.
Rothwell, S. W., Grasser, W. A., & Murphy, D. B. (1986) *J. Cell Biol. 102*, 619–627.
Roychowdhury, S., & Gaskin, F. (1988) *Biochemistry 27*, 7799–7805.
Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.
Weeds, A. (1982) *Nature 296*, 811–815.
Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1858–1862.
Weisenberg, R. D., Deery, W. J., & Dickinson, P. J. (1976) *Biochemistry 15*, 4248–4254.
Zeeberg, B., & Caplow, M. (1981) *J. Biol. Chem. 256*, 12051–12057.

Kinetics of Interleukin 2 mRNA and Protein Produced in the Human T-Cell Line Jurkat and Effect of Cyclosporin A

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Received June 1, 1988; Revised Manuscript Received October 14, 1988

ABSTRACT: The kinetics of interleukin 2 mRNA accumulation in the leukemic T-cell line Jurkat, which can be induced with phytohemagglutinin and phorbol 12-myristate 13-acetate to produce large amounts of interleukin 2, was analyzed by a modified DNA-excess solution hybridization assay using a 5′-^{32}P-labeled oligodeoxyribonucleotide 30 bases long as probe. Cyclosporin A was used as a valuable tool to gain more insight into the quantitative aspects of interleukin 2 production, on the basis of the assumption that transcription of the interleukin 2 gene is completely inhibited shortly after administration of cyclosporin A. The half-life of interleukin 2 mRNA was estimated to be approximately 2 h. With the aid of simple mathematical models, we have been able to relate the concentration of interleukin 2 protein in the supernatant to the interleukin 2 mRNA kinetics. This novel quantitative kinetic analysis revealed that, independent of the absence or presence of cyclosporin A, interleukin 2 protein is synthesized at a rate of approximately 1.3 molecules per molecule of interleukin 2 mRNA per second and secreted within 2 h after it is synthesized and that its half-life in the supernatant is approximately 10 h.

Interleukin 2 (IL2),[1] or T-cell growth factor, has been recognized as a key immunoregulatory protein for the proliferation of activated T-cells of various types. Studies of the control of IL2 expression in T-cells have revealed that IL2 production is induced at the transcriptional level upon antigenic or mitogenic stimulation. Moreover, it has been shown that cyclosporin A (Sandimmune, CsA), a powerful immunosuppressive drug of considerable clinical importance, inhibits the expression of the IL2 gene (Arya & Gallo, 1987; Kaempfer et al., 1987; Paetkau et al., 1987).

In order to gain more insight into the quantitative aspects of IL2 production, we have studied the kinetics of IL2 mRNA and secreted IL2 protein produced in the human T-cell line Jurkat and the influence of CsA added at different times after induction. To assay IL2 mRNA, we have modified existing solution hybridization methodology and developed a versatile method using a chemically synthesized 5′-^{32}P-labeled oligodeoxyribonucleotide as single-stranded probe. In vitro synthesized IL2 mRNA served as a hybridization standard and was also used to evaluate the sensitivity of the assay. By a novel approach, applying simple mathematical models, we have been able to estimate from our kinetic data the absolute rates of IL2 mRNA production and IL2 protein synthesis, as well as the half-lives of both species. The general applicability of using our assay method and models for studying the kinetics of other mRNA species is discussed, and criteria for choosing suitable oligonucleotide probes by computer analysis of mRNA sequences are offered.

Materials and Methods

Stimulation of Jurkat Cells and Determination of IL2 Protein in the Supernatant. Jurkat leukemic T cells (subclone K16) were grown in RPMI 1640, containing 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100

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[1] Abbreviations: CsA, cyclosporin A; HLA-B, major histocompatibility antigen B; IL2, interleukin 2; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; AUC, area under the curve.

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μg/mL streptamycin, 50 μM β-mercaptoethanol, and 25 mM sodium bicarbonate. For induction, phytohemagglutinin (PHA, 1 μg/mL) and phorbol 12-myristate 13-acetate (PMA, 20 ng/mL) were added to cells at a density of 2×10^6 cells/mL of serum-free medium. Cells were treated for different time intervals with CsA (1 μg/mL = 8×10^{-7} M). Viable cells were determined after recovery and the cells stored as pellets at −80 °C. Supernatants were assayed for IL2 production by measuring [^{3}H]thymidine incorporation into IL2-dependent CTLL cells (Robb, 1982). The value of [^{3}H]thymidine incorporation obtained with PMA at the concentration used for induction of the Jurkat cells was subtracted as background from each value. Primary data (cpm/mL) were converted into pmol/mL by use of recombinant IL2 as standard.

RNA Extraction. Total RNA from Jurkat cells was isolated by extraction with guanidinium thiocyanate (Chirgwin et al., 1979) and centrifugation through $CsCl_2$ (Glisin et al., 1974). Purified RNA was stored in 10 mM Tris, pH 7.5–1 mM EDTA at −70 °C. Total RNA concentration was determined spectrophotometrically at 260 nm; an absorption of 1.0 in a 1-cm path-length cuvette is equivalent to 40 μg/mL RNA. The values of total RNA concentrations served to calculate the amount of total RNA per cell, assuming a recovery of 100%.

Synthesis, Purification, and Labeling of the 5′-Hydroxy Terminus of the Oligodeoxyribonucleotide Probes. The following oligonucleotide probes complementary to portions of the mRNAs for human IL2 (Taniguchi et al., 1983) and the major histocompatibility antigen B (HLA-B) (Sood et al., 1981) were synthesized on an automatic DNA synthesizer (Applied Biosystems, 380A) using phosphoramidite chemistry and purified by gel electrophoresis on polyacrylamide gels under denaturing conditions:

oIL2/30:

5′-GGTTGCTGTCTCATCAGCATATTCACACAT

oHLA-B/33:

5′-GCTGTACATGGCATGTGTATCTCTGCTCTTCTC

Purified oligonucleotides (4 pmol) were labeled at the 5′-end with polynucleotide kinase and [γ-^{32}P]ATP under standard kinase conditions (Maniatis et al., 1982), separated from unincorporated ATP by gel filtration through Sephadex G-25, precipitated overnight at −20 °C, redissolved in 100 μL of 10 mM Tris, pH 7.5–1 mM EDTA, and stored at 4 °C. Specific activity of the probes was approximately 10^4 cpm/fmol.

Northern Blot Hybridization. Total RNA was denatured with glyoxal, size-fractioned by electrophoresis (80 V, 4 h) on a 1% agarose gel, and transferred to GeneScreen membranes (NEN) in 20× SSC (1× SSC = 150 mM NaCl and 15 mM sodium citrate) (Thomas, 1980). Hybridization conditions were adopted from Singh and Jones (1984): Blots were prehybridized in 4× SET (1× SET = 0.15 M NaCl, 20 mM Tris, pH 7.5, 1 mM EDTA), 0.1% sodium pyrophosphate, 0.2% SDS, and 200 μg/mL heparin sodium salt at 68 °C for 2 h. After addition of the oligonucleotide probe (approximately 2×10^7 cpm, 10^7 cpm/pmol), blots were hybridized at 68 °C for 2 h. Blots were then washed several times in 2× SET–0.2% SDS: 5 min at room temperature, twice for 30 min at 68 °C, and finally 5 min at room temperature. After being dried, blots were exposed to Fuji RX X-ray film with an intensifier screen for 1–4 days.

In Vitro Synthesis of IL2 mRNA. The recombinant plasmid pGEM-1/IL2 was constructed by inserting a human IL2 cDNA [kindly provided by Dr. Steven C. Clark, Genetics Institute, Boston (Clark et al., 1984)] into the *Pst*I site of pGEM-1 (Promega Biotec). The orientation of the insert was determined by restriction analysis with *Rsa*I, and a clone (pGEM-1/IL2) was selected to allow the synthesis of IL2 mRNA by T7 RNA polymerase. Plasmid pGEM-1/IL2 was linearized close to the 3′-end of the cDNA insert by digestion with *Sty*I, extracted with phenol, and precipitated with ethanol. The linear DNA template (5 μg, 50 μg/mL) was transcribed in 40 mM Tris, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, RNasin (2.4 units/μL), and 500 μM each ribonucleotide triphosphate. A total of 60 units of T7 RNA polymerase was added for a 2-h synthesis at 37 °C. Following RNA synthesis the DNA template was removed by incubation with 5 units of DNase RQ1 at 37 °C for 15 min. The reaction mixture was extracted with phenol, and RNA was precipitated with ethanol, redissolved in 10 mM Tris, pH 7.5–1 mM EDTA, and separated from unincorporated ribonucleotide triphosphates by centrifugation through a membrane of a Centricor microconcentrator (Amicon, cutoff 30 000). Electrophoresis of a sample on a 5% polyacrylamide gel (7 M urea) and staining with ethidium bromide showed a single band of the appropriate size of approximately 800 bases. The yield of the RNA transcript was determined spectrophotometrically at 260 nm (7.5 μg) and its molar concentration calculated by assuming a molecular weight of 2.73×10^5.

Measurement of mRNA by Solution Hybridization/S1 Nuclease Digestion. Duplicate samples of total RNA (10–15 μg) were hybridized with (1–2) × 10^5 cpm of the appropriate 5′-end-labeled probe in a total volume of 30 μL of 40% formamide, 0.6 M sodium chloride, 10 mM Tris, pH 7.4, and 4 mM EDTA for 24 h at 37 °C. Duplicate 10-μL samples of each hybridization mixture were added to 90 μL of ice-cooled S1 buffer (0.3 M sodium acetate, pH 5.0, 0.3 M sodium chloride, 3 mM zinc sulfate, 25 μg/mL sonicated herring sperm DNA) and thoroughly mixed by vortexing. Equal distribution of probe was analyzed by scintillation spectrophotometry of 45 μL of each sample in 2.5 mL of scintillation fluid (Hionic-Fluor, United Technologies Packard). S1 nuclease (100 units in 2 μL of S1 buffer) was added to the remaining 55 μL of each sample, followed by incubation at 15 °C for 5 h. A total of 45 μL was spotted onto Whatman DE-81 ion-exchange paper disks (2.3-cm diameter). The disks were washed four times with 0.5 M phosphate buffer, pH 6.5, at room temperature (15, 2, 2, and 1 min) and twice in deionized water (1 and 1 min) and rinsed twice with 95% ethanol. The disks were dried at 65 °C, transferred individually into scintillation vials, and covered with 7 mL of scintillation fluid (Luma Gel, Lumac/3M bv). Counts were accumulated for 10–20 min per vial. Background values were determined in samples with total RNA extracted from rat pituitary and subtracted from each value. Primary data (cpm/μg of total RNA) were converted to cpm/cell, by use of the measured values of recovered total RNA and number of viable cells per cell culture or additionally into molecules per cell, with an in vitro synthesized IL2 mRNA as standard.

Results and Discussion

Development of a Modified Solution Hybridization Method with Oligonucleotides as Probes. The most important aspect of any hybridization assay is the discrimination and separation of hybridized from nonhybridized probe. In DNA-excess solution hybridization with single-stranded probes discrimination is accomplished enzymatically by S1 nuclease. After the enzymatic reaction, separation is normally achieved either by trichloroacetic acid precipitation and absorption to G/C filters (Williams et al., 1986) or by gel electrophoresis (Maniatis et al., 1982). However, in RNA-excess solution

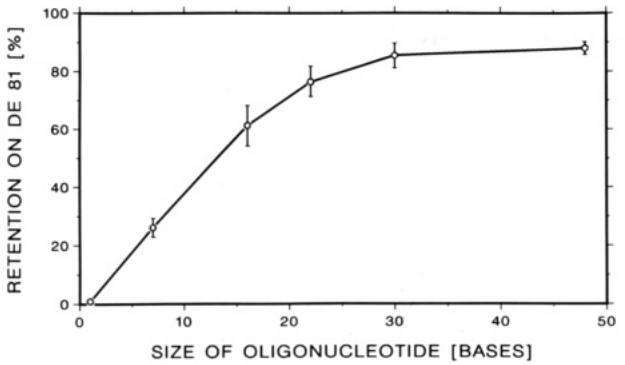

FIGURE 1: Retention of 5′-^{32}P-labeled oligonucleotides on Whatman DE-81 ion-exchange paper disks as function of size of oligonucleotide. Quadruplicates of approximately 5×10^4 cpm of a labeled mononucleotide ([γ-^{32}P]ATP) and 5′-labeled oligonucleotides of different length (7, 16, 25, 30, and 48 bases) in 45 μL of S1 buffer were applied to Whatman DE-81 ion-exchange paper disks (diameter 2.3 cm). Two disks each were washed four times with 0.5 M phosphate buffer, pH 6.5, at room temperature (15, 2, 2, and 1 min) and twice in deionized water (1 and 1 min) and rinsed twice with 95% ethanol. The remaining two disks each were not washed. All disks were dried at 65 °C and measured by scintillation spectrometry in 7 mL of scintillation fluid (Luma Gel, Lumac/3M bv). The retained radioactivity after washing is shown as the ratio of unwashed control disks (mean of two values with standard deviation).

hybridization (R_0t analysis), Whatman DE-81 ion-exchange paper disks have been used successfully for separation of intact from digested cDNA probe (Wegnez et al., 1982). We assumed that the latter separation method should also be applicable after DNA-excess solution hybridization with an oligonucleotide as probe. Therefore, we have analyzed to what extent chemically synthesized oligodeoxyribonucleotides are retained on these disks after washing with 0.5 M phosphate buffer. From the results, shown in Figure 1, we conclude that these disks can be used efficiently after DNA-excess solution hybridization/S1 nuclease digestion provided that probes of 30 bases in length or longer are used; for these probes retention of intact probe is >85%. The finding that even an oligonucleotide of 48 bases in length is not retained completely may be explained by the presence of residual unincorporated [γ-^{32}P]ATP. Less than 0.5% of radioactivity is retained after washing, if an oligonucleotide of 30 bases in length is previously digested with S1 nuclease.

In order to evaluate scope and limitations of the modified solution hybridization assay, we performed several control experiments. First, the oligonucleotide probes for IL2 and HLA-B mRNA were characterized in a Northern blot, shown in Figure 2. Both probes hybridized to specific mRNA species of the expected size. Then, we measured the accumulation of IL2 mRNA and HLA-B mRNA in induced Jurkat cells, treated with different doses of CsA, with the modified solution hybridization assay. The results, shown in Figure 3, demonstrate the dose-dependent inhibition of IL2 mRNA accumulation by CsA, whereas the mRNA of HLA-B was only affected at the highest dose of CsA. Both results are consistent with earlier findings from Northern hybridization analysis (Kroenke et al., 1984).

The products of the oligonucleotide probe for IL2 mRNA corresponding to bar B of Figure 3 were analyzed by polyacrylamide gel electrophoresis, which showed clearly that the radioactivity retained on the disks was almost exclusively derived from full-length oligonucleotide probe.

Finally, we have evaluated the sensitivity of the new method by hybridization of the oligonucleotide probe for IL2 mRNA to a serial dilution of in vitro synthesized IL2 mRNA. The results are shown in Figure 4. The minimal amount of the

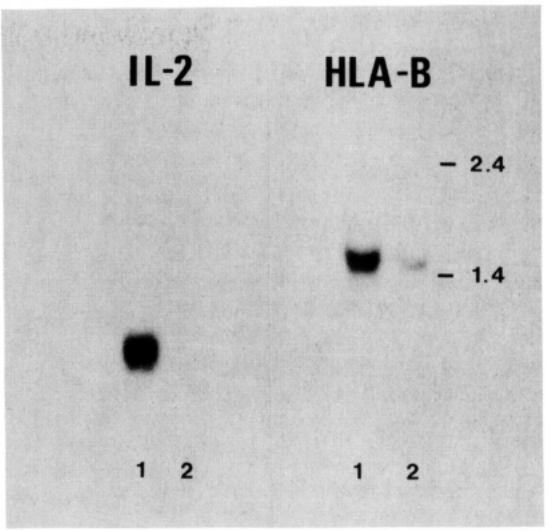

FIGURE 2: Northern blot analysis of IL2 and HLA-B mRNA. Total RNA was denatured and size fractionated by electrophoresis in a 1% agarose gel. Following transfer to GeneScreen, blots were hybridized with 5′-^{32}P-labeled oligonucleotide probes for IL2 and HLA-B, respectively. After being washed, blots were exposed to X-ray film with an intensifier screen for 1 (HLA-B) or 4 days (IL2). Lane 1, 20 μg of total RNA from Jurkat cells, 6 h after induction with PHA and PMA. Lane 2, 20 μg of total RNA from noninduced Jurkat cells. Commercially available RNA markers (BRL) were used to indicate fragment size in kilobases.

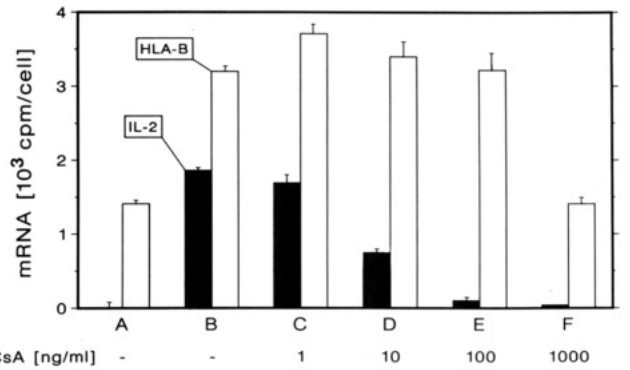

FIGURE 3: IL2 and HLA-B mRNA in Jurkat cells, 6 h after induction, as a function of the dose of CsA added at the beginning of the induction. (A and B) Noninduced and induced controls with no CsA. (C–F) Induced cells with CsA concentrations as indicated. IL2 mRNA and HLA-B mRNA contents were analyzed by solution hybridization/S1 nuclease digestion and expressed as cpm/cell, as described under Materials and Methods. Error bars represent standard errors of the mean of four values.

in vitro synthesized IL2 mRNA detected was 0.08 fmol (4.8×10^7 molecules, 22 pg) per hybridization (response = 2 × background), and the measured values showed a linear relationship from 0.1 to 10 fmol per hybridization.

Kinetics of IL2 mRNA Accumulation and Secreted IL2 Protein in Induced Jurkat Cells and Its Inhibition by CsA. Initially, we measured IL2 mRNA and IL2 protein at 6 h after induction with PHA and PMA, with CsA (1 μg/mL = 8×10^{-7} M) added at different times. The results, shown in Figure 5, demonstrate that the earlier CsA is added the stronger IL2 mRNA and protein concentrations are decreased by CsA. If CsA is added less than 2 h after induction, IL2 protein is undetectable in the supernatant at 6 h after induction. If CsA is added 4 or 5 h after induction, only a slight reduction of IL2 mRNA and no significant effect on IL2 protein is detected at 6 h after induction. We then measured IL2 mRNA and protein as a function of time (1–24 h) after induction in the absence of CsA and with CsA added at 4 and 5 h after in-

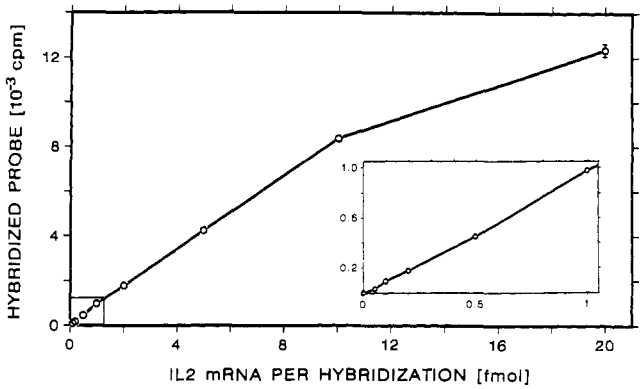

FIGURE 4: Response of the solution hybridization/S1 nuclease digestion assay as a function of known amounts of in vitro synthesized IL2 mRNA. Serial dilutions (0.05–20 fmol) of in vitro synthesized IL2 mRNA were mixed with 5 μg of total RNA extracted from rat pituitary as carrier and analyzed by solution hybridization/S1 nuclease digestion. Hybridized probe is shown as cpm vs fmol of IL2 mRNA per hybridization. The lower amounts are shown on the insert. The response obtained with no IL2 mRNA added has been subtracted as background from each value. Only the largest error bar is shown, representing the standard error of the mean of four values. The minimal amount of the in vitro synthesized IL2 mRNA detected was 0.08 fmol (4.8×10^7 molecules, 22 pg) per hybridization (response = 2 × background).

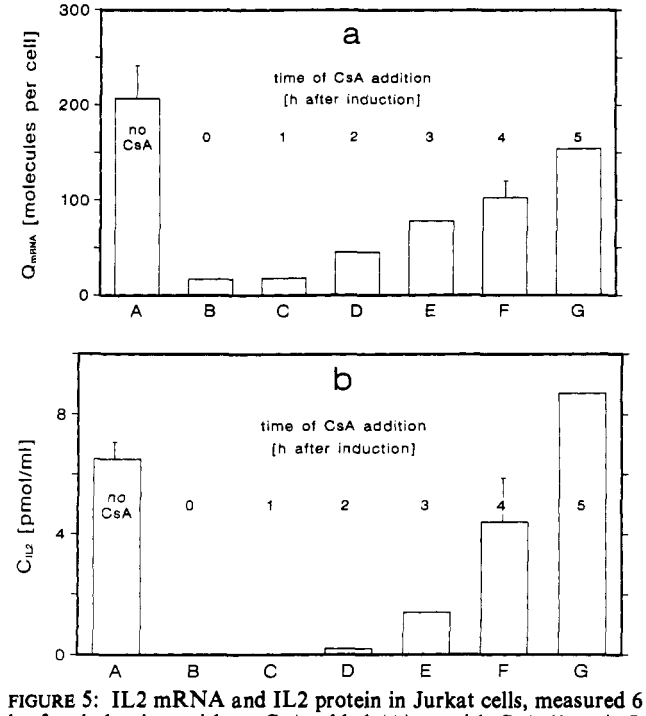

FIGURE 5: IL2 mRNA and IL2 protein in Jurkat cells, measured 6 h after induction, with no CsA added (A) or with CsA (1 μg/mL = 8×10^{-7} M) added at the times indicated (B–G). (a) IL2 mRNA content (Q_{mRNA}) analyzed by solution hybridization/S1 nuclease digestion and expressed as molecules per cell, as described under Materials and Methods. (b) IL2 protein concentration (C_{IL2}) in the supernatant expressed as pmol/mL, as described under Materials and Methods. Values in both figures are from single-induction experiments (B–E, G) or means of three (A) or two (F) independent experiments with error bars representing the standard errors of the mean.

duction, as illustrated in Figure 6. In the absence of CsA, detectable levels (14 molecules/cell) of IL2 mRNA are found 2 h after induction with PHA and PMA; maximal levels are measured at 5–6 h, followed by a slow decrease to about 50% of maximal levels over the next 18 h. Administration of CsA 4 or 5 h after induction is followed quickly by a rapid decrease of IL2 mRNA in the cells. In contrast to the mRNA kinetics, the concentration of IL2 protein shows a lag time of about 3–4

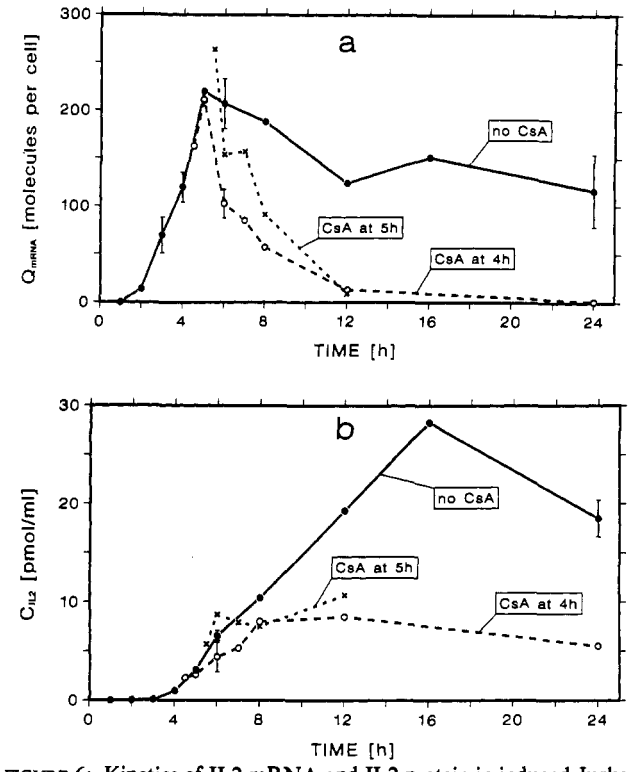

FIGURE 6: Kinetics of IL2 mRNA and IL2 protein in induced Jurkat cells with no CsA added (●) or with CsA (1 μg/mL = 8×10^{-7} M) added 4 (○) or 5 h (×) after induction. (a) IL2 mRNA content (Q_{mRNA}) analyzed by solution hybridization/S1 nuclease digestion and expressed as molecules per cell, as described under Materials and Methods. (b) IL2 protein concentration (C_{IL2}) in the supernatant expressed as pmol/mL, as described under Materials and Methods. In both figures, symbols without error bars represent data from single-induction experiments. Symbols with error bars (standard errors of the mean) represent the mean of two to three independent induction experiments.

h and in the absence of CsA increases steadily, reaching an apparent peak at 16 h after induction. When CsA is added 4 or 5 h after induction, the increase of protein concentration is blunted shortly thereafter.

Quantitative Analysis of the Kinetic Data. Data of IL2 mRNA concentration and values of its apparent half-life in T-cells in the absence or presence of CsA have been reported (Arya & Gallo, 1987; Clark et al., 1984; Kaempfer et al., 1987; Paetkau et al., 1987). However, our data allow a novel and deeper insight into the kinetics of IL2 mRNA and protein production by applying simple mathematical models. In analogy with pharmacokinetic theory (Gibaldi & Perrier, 1982), the time dependence of the IL2 mRNA content per cell [denoted $Q_{mRNA}(t)$] can be related to the mRNA production rate [denoted $\dot{P}_{mRNA}(t)$] and the mRNA degradation rate which we assume to be a first-order process with rate constant k_{deg}^{mRNA}, according to

$$dQ_{mRNA}/dt = \dot{P}_{mRNA}(t) - k_{deg}^{mRNA} Q_{mRNA}(t) \quad (1)$$

If it is further assumed that CsA totally suppresses IL2 mRNA production shortly after its addition ($\dot{P}_{mRNA}(t) = 0$), then $Q_{mRNA}(t)$ should thereafter decay exponentially with a half-life given by

$$t_{1/2}(\text{mRNA}) = \ln 2 / k_{deg}^{mRNA} \quad (2)$$

As seen from the semilogarithmic plot of Figure 7, the decay of the IL2 mRNA levels after CsA addition at 4 and 5 h is approximately linear (e.g., exponential) with half-lives (derived by least-squares analysis) of 2.11 and 1.73 h, respectively. In contrast, in the absence of CsA, the mRNA levels decay with

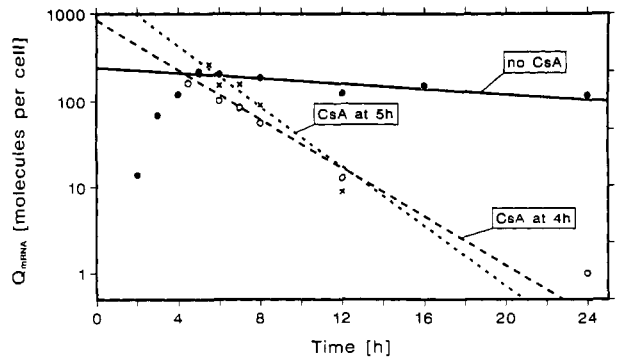

FIGURE 7: Half-life estimation of IL2 mRNA in induced Jurkat cells, with no CsA added (●) or with CsA (1 μg/mL = 8×10^{-7} M) added 4 (○) or 5 h (×) after induction. Data points of the descending phases of the curves in Figure 6a were fit to a single-exponential decay by least-squares analysis (solid and dashed lines). The apparent half-lives of IL2 mRNA were 18.8 h in the absence of CsA, 2.11 h with CsA added 4 h after induction, and 1.73 h with CsA added 5 h after induction.

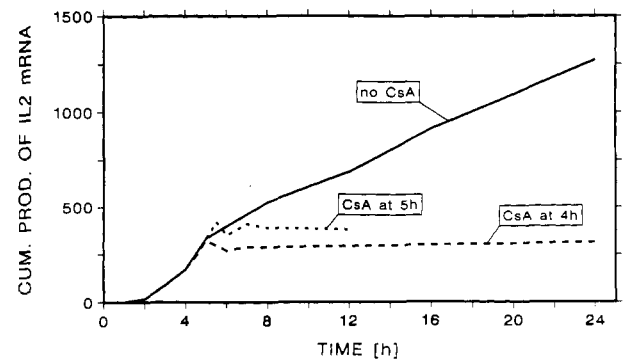

FIGURE 8: Estimated cumulative IL2 mRNA production (molecules per cell) in the absence or presence of CsA (1 μg/mL = 8×10^{-7} M). Cumulative production was determined from eq 3, assuming that the actual half-life of IL2 mRNA was ca. 2 h, independent of the absence or presence of CsA. Between 5 and 24 h, the untreated system produces approximately 50 IL2 mRNA molecules per cell per hour, about half the rate of the preceding period. For the curves of the treated systems, the cumulative production prior to CsA addition was taken to be equal to that of the untreated system. Following addition of CsA, no further production of IL2 mRNA is observed as a consequence of the assumptions used in the data analysis.

an apparent half-life of 18.8 h. All of these values are consistent with the wide range of mRNA half-lives in eukaryotic cells (Braverman, 1987). Moreover, they are consistent with the reported values of the apparent half-life of IL2 mRNA in Jurkat cells in the absence of CsA (Clark et al., 1984) or in the mouse T lymphoma cell line EL4.E1 in the presence of CsA (Paetkau et al., 1987). If the actual degradation half-life of IL2 mRNA is not affected by CsA (e.g., ca. 2 h), then the slow decay of the untreated cells must reflect the persistence of mRNA production during the entire 24 h of our experiment. Taking k_{deg}^{mRNA} to be a constant (ca. 0.365 h^{-1} = ln 2/1.9 h), independent of CsA addition, the cumulative amount of mRNA produced as a function of time t can be deduced by numerical integration of eq 1, in analogy with the Wagner–Nelson equation of pharmacokinetics (Gibaldi & Perrier, 1982):

$$\text{cumulative mRNA production} = \int_0^t \dot{P}_{mRNA}(t')\,dt' = Q_{mRNA}(t) + k_{deg}^{mRNA}\int_0^t Q_{mRNA}(t')\,dt' \quad (3)$$

Figure 8 illustrates the cumulative mRNA production estimated from eq 3, for all three treatment conditions. Between 5 and 24 h, the untreated system produces approximately 50 mRNA molecules per cell per hour, about half the rate of the preceding period. By our assumption, the treated systems show no production after CsA addition. Alternatively, if k_{deg}^{mRNA} is influenced (increased) by CsA, a possibility that cannot be excluded from our data nor on theoretical grounds (Darnell, 1982), the cumulative production would be less than that shown in Figure 8.

Similar to eq 1, we may express the time dependence of the IL2 protein concentration in the supernatant [denoted $C_{IL2}(t)$] in terms of the rate of protein secretion per cell [denoted $Sec_{IL2}(t)$], the degradation rate constant of the IL2 protein in the supernatant k_{deg}^{IL2}, the number of cells (N_0), and the volume of the supernatant (V), as

$$\frac{dC_{IL2}}{dt} = \frac{N_0}{V}Sec_{IL2}(t) - k_{deg}^{IL2}C_{IL2}(t) \quad (4)$$

We now assume that $Sec_{IL2}(t)$ corresponds to the rate of IL2 synthesis at an earlier time, e.g., $Syn_{IL2}(t - T_0)$ (where T_0 is the lag time prior to secretion), and further that $Syn_{IL2}(t - T_0)$ is proportional to the IL2 mRNA content of the cell at that time:

$$Syn_{IL2}(t - T_0) = k_{trans}Q_{mRNA}(t - T_0) \quad (5)$$

k_{trans} is a rate constant for protein translation per mRNA molecule. The relationship between C_{IL2} and $Q_{mRNA}(t - T_0)$ will thus be given by

$$\frac{dC_{IL2}}{dt} = \left(\frac{N_0}{V}k_{trans}\right)Q_{mRNA}(t - T_0) - k_{deg}^{IL2}C_{IL2}(t) \quad (6)$$

Integrating eq 6, we obtain

$$C_{IL2}(t) = \left(\frac{N_0}{V}k_{trans}\right)\int_0^t Q_{mRNA}(t' - T_0)\,dt' - k_{deg}^{IL2}\int_0^t C_{IL2}(t')\,dt' \quad (7)$$

In essence, eq 7, the key result of our model, predicts that $C_{IL2}(t)$ should be proportional to the area under the curve (AUC) of Q_{mRNA} evaluated up to time $t - T_0$, with a correction related to the AUC of $C_{IL2}(t)$, itself. To test this prediction numerically, we have performed a multiple regression analysis on all available data from the three treatment conditions (no CsA, CsA added at 4 and 5 h), using the regression equation

$$C_{IL2}(t) = \lambda_1 AUC_{mRNA}(t) + \lambda_2 AUC_{IL2}(t) + \lambda_3 \quad (8)$$

Although for computational reasons the lag time parameter (T_0) has been neglected in eq 8, its absence is compensated to some extent by the constant term λ_3 (see discussion below). Figure 9 illustrates the result of fitting eq 8 to the data. All three data sets superimpose well on the regression line, whose correlation coefficient is 0.933. The deduced parameter values (±SD) are

$$\lambda_1 = 0.016\ (\pm 0.002)\ \frac{pmol_{IL2}\cdot cell}{mL\cdot molecules_{mRNA}\cdot h} \quad (9a)$$

$$\lambda_2 = -0.073\ (\pm 0.019)\ h^{-1} \quad (9b)$$

$$\lambda_3 = -1.044\ pmol_{IL2}/mL \quad (9c)$$

By comparison of eq 7 and 8, we may determine the translational rate constant k_{trans} and the half-life of IL2 protein from the regression parameters as

$$k_{trans} = \frac{\lambda_1 V}{N} = 1.3\frac{molecules_{IL2}}{molecules_{mRNA}\cdot s} \quad (10a)$$

$$t_{1/2}(IL2) = \ln 2/\lambda_2 = 9.5\ h \quad (10b)$$

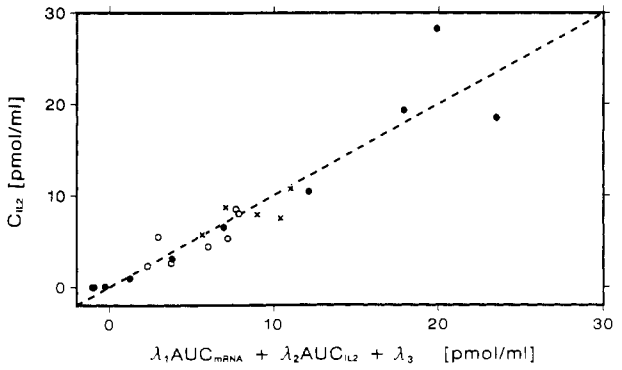

FIGURE 9: Relation of IL2 protein concentration (C_{IL2}) at different time points to the area under the curve (AUC) of the mRNA kinetics and the AUC of IL2 protein kinetics on the basis of eq 8. A multiple regression analysis was performed with all available data (Figure 6) from the three treatment conditions [no CsA (●); CsA (1 μg/mL = 8×10^{-7} M) added 4 (○) and 5 h (×) after induction]. AUC_{mRNA} and AUC_{IL2} were evaluated up to the corresponding time points of each C_{IL2} measurement. For the treated systems, the AUC values at the time points prior to CsA addition were taken from the curves of the untreated system. Parameter values derived are as follows: $\lambda_1 = 0.016$ (±0.002) [$pmol_{IL2}$·cell/$molecules_{IL2mRNA}$·mL·h]; $\lambda_2 = -0.073$ (±0.019) [h^{-1}]; $\lambda_3 = -1.044$ [$pmol_{IL2}$/mL]. All three data sets are consistent with the line of identity [(--) $r = 0.933$] with the same set of regression parameters, which implies that translation, posttranslational processing, secretion, and stability of IL2 protein are not affected by CsA.

Furthermore, the negative sign of λ_3 is consistent with the expected compensation for the AUC_{mRNA} term, which has been evaluated to time t, rather than time $t = T_0$ (see eq 7 and 8). Taking the ratio λ_3/λ_1 yields a correction to the AUC_{mRNA} term of −65 molecules·h/cell. This correction, which is only important during the initial phase of the mRNA kinetics (Figure 6a), is approximately equal to the AUC_{mRNA} between the 1- and 3-h time points. This 2-h interval, during which no IL2 protein was measurable in the supernatant (Figure 6b), can thus be regarded as an estimate for the approximate lag time (T_0) between IL2 synthesis and secretion.

The consistency of all three data sets with the same set of parameters implies that the coupling of IL2 protein synthesis to the mRNA levels, as well as the secretion and stability of the protein, is not influenced by the addition of CsA.

It is interesting to consider the magnitude deduced for the translational rate constant k_{trans} and its possible interpretation. In principle, either initiation, elongation, or termination could constitute the rate-limiting step for protein synthesis (Bergmann & Lodish, 1979; Von Heijne et al., 1987). As an upper limit, k_{trans} should be equal to the maximal number of active ribosomes per mRNA (N_R) multiplied by their maximal speed of movement down the mRNA chain (V_R):

$$k_{trans} = N_R V_R \tag{11}$$

In *Escherichia coli*, at maximum utilization, N_R corresponds to about 1 ribosome every 80 nucleotides, whereas a 400 amino acid protein (1200 nucleotides) requires 10 s for synthesis (e.g., V_R = 120 nucleotides/s) (Watson, 1970). Together, these values yield an upper limit of k_{trans} (in *E. coli*) of

$$k_{trans} = \frac{1}{80 \text{ nucleotides}} \frac{120 \text{ nucleotides}}{\text{s}} = 1.5 \text{ s}^{-1} \tag{12}$$

This value is remarkably close to our experimental estimate of k_{trans} for the stimulated T-cell line Jurkat, indicating that the observed rates of IL2 synthesis are comparable to the maximal ones possible in *E. coli*. In comparison to the rate of protein synthesis in rabbit reticulocytes (Bergmann & Lodish, 1979), IL2 production appears to be more than an order of magnitude faster. Deductions of k_{trans} for other species of mRNA and their protein products (including viral proteins) in stimulated T-cells and other eukaryotic cells would be of great value in further elucidating the rate-controlling mechanisms of protein synthesis.

General Application of 5′-^{32}P-Labeled Oligodeoxyribonucleotides in DNA/mRNA Hybridization Assays. Regulation of specific mRNA accumulation has been recognized as an important aspect of the pharmacological action of a variety of drugs. Examples are bromocriptin which inhibits accumulation of prolactin mRNA (Brocas et al., 1981; Maurer, 1982), mevinolin which induces the mRNA for the low-density lipoprotein receptor (Ma et al., 1986), and cyclosporin A which inhibits the induction of several mRNAs in T-lymphocytes (Granelli-Piperno et al., 1984). An important tool for such analyses is the determination of specific mRNA concentrations by nucleic acid hybridization. Whereas Northern blot analysis is very useful for qualitative investigations, solution hybridization methods (DNA excess and RNA excess) are more suitable for accurate quantitative assessments (Williams et al., 1986; Quarless & Heinrich, 1986). A prerequisite for solution hybridization is the availability of single-stranded probes. Several biological methods for preparing single-stranded cDNA or cRNA are available (Williams et al., 1986; Quarless & Heinrich, 1986). However, chemical synthesis of oligodeoxyribonucleotides is clearly the most versatile and straightforward method for preparing short lengths of single-stranded DNA. Surprisingly, such probes have been used only rarely in solution hybridization assays (Carr et al., 1985; Ornitz et al., 1985). Here, we suggest how oligodeoxyribonucleotides can be used efficiently as probes in a DNA-excess solution hybridization assay. First, probes of 30 bases or slightly longer are required to allow the use of Whatman DE-81 ion-exchange paper disks for separation of intact from digested probe. Using these disks, we have found that background levels are usually <0.5% of total probe added. Second, we suggest the following empirically derived criteria for choosing a suitable oligonucleotide probe:

(1) The probes should correspond to a region of mRNA that is free of double-stranded secondary RNA structure.

(2) The probes should not self-hybridize. Self-hybridization creates two potential problems: (a) incomplete digestion of excess probe by S1 nuclease occurs; (b) [^{32}P]phosphate incorporation by polynucleotide kinase is reduced when the 5′-end of the probe self-hybridizes.

(3) The last two bases of the 5′-end should be G or C in order to increase the strength of base pairing at the position of the radioactive label.

To apply these criteria, we have used the SEQ computer program developed by Intelligenetics Inc. (Palo Alto, CA).

DNA-excess solution hybridization offers several advantages: First, the assay requires only small amounts of total RNA. Second, it is carried out in solution so that essentially all the mRNA and the probe are available to react. Third, DNA-excess solution hybridization follows pseudo-first-order kinetics, so that eq 13 can be applied. This equation implies

$$C_{hyb} = C_{mRNA}(t_0)(1 - e^{-kC_{oligo}(t_0)t}) \tag{13}$$

the following: First, at any time the concentration of hybrid (denoted C_{hyb}) is a function only of the initial concentrations of mRNA and probe [denoted $C_{mRNA}(t_0)$ and $C_{oligo}(t_0)$]. Second, the sensitivity of the assay depends on the degree of hybridization, which is a function of the stability of the hybrid, $C_{oligo}(t_0)$, and the hybridization time.

In addition to the oligonucleotide probes described in this paper, we have used successfully a variety of oligonucleotides

to measure the accumulation of other mRNAs. All these probes have been chosen according to the criteria listed above, and hybridizations have been performed with the same conditions as with the probe for IL2 mRNA. So far, the large excess of unhybridized oligonucleotides was always efficiently digested by S1 nuclease under the same mild conditions (15 °C at pH 5.0, 5 h).

We believe that this assay with 5′-^{32}P-labeled oligonucleotides as probes is practical and versatile. Measurement of specific mRNA concentrations is accessible with published sequencing data, but without need for a cloned cDNA as starting material, if relative values are sufficient. For determination of absolute mRNA values, a hybridization standard derived from a cloned cDNA must be included.

We anticipate that the discovery of novel modulators of specific gene expression will allow exploration of the interrelationship between mRNA and protein kinetics for a variety of genes. Studying these quantitative aspects with our assay method and kinetic models will provide us with a deeper understanding of gene regulation in vitro and in vivo.

Acknowledgments

We thank Dr. Steven C. Clark for supplying a plasmid containing the cDNA of human IL2. R.N. acknowledges the hospitality of Prof. John D. Baxter (The Metabolic Research Unit, University of California, San Francisco) and helpful suggestions by Dr. David G. Gardner and Dr. Barry J. Gertz during a sabbatical leave from Sandoz.

Registry No. CsA, 59865-13-3.

References

Arya, S. K., & Gallo, R. C. (1987) in *Molecular Cloning and Analysis of Lymphokines* (Webb, D. R., & Goeddel, D. V., Eds.) pp 35–57, Academic Press, New York.

Bergmann, J. E., & Lodish, H. F. (1979) *J. Biol. Chem. 254*, 11927–11937.

Braverman, G. (1987) *Cell 48*, 5–6.

Brocas, H., van Coevorden, A., Seo, H., Refetoff, S., & Vassart, G. (1981) *Mol. Cell. Endocrinol. 22*, 25–30.

Carr, F. E., Ridgway, E. Ch., & Chin, W. W. (1985) *Endocrinology 117*, 1272–1278.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Clark, St. C., Arya, S. K., Wong-Staal, F., Matsumoto-Kobayashi, M., Kay, R. M., Kaufman, R. J., Brown, E. L., Shoemaker, Ch., Copeland, T., Oroszlan, St., Smith, K., Sarngadharan, M. G., Linder, St. G., & Gallo, R. C. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2543–2547.

Darnell, J. E., Jr. (1982) *Nature 297*, 365–371.

Gibaldi, M., & Perrier, D. (1982) *Pharmacokinetics*, 2nd ed., Marcel Dekker, New York.

Glisin, V., Crkvenjakov, R., & Byus, C. (1974) *Biochemistry 13*, 2633–2637.

Granelli-Piperno, A., Inaba, K., & Steinmann, R. M. (1984) *J. Exp. Med. 160*, 1792–1802.

Kaempfer, R., Efrat, S., & Marsh, S. (1987) in *Molecular Cloning and Analysis of Lymphokines* (Webb, D. R., & Goedell, D. V., Eds.) pp 59–72, Academic Press, New York.

Kroenke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, Th. A., & Greene, W. C. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5214–5218.

Ma, P. T. S., Gil, G., Sudhof, T. C., Bilheimer, D. W., Goldstein, J. L., & Brown, M. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8370–8374.

Maniatis, T., Fritsch, E. F., & Sambrock, J. (1982) *Molecular Cloning*, Cold Spring Harbor Press, Cold Spring Harbor, NY.

Maurer, R. A. (1982) in *Cellular Regulation of Secretion and Release* (Conn, P. M., Ed.) pp 267–300, Academic Press, New York.

Ornitz, D. M., Palmiter, D. R., Hammer, R. E., Brinster, R. L., Swift, G. H., & MacDonald, R. J. (1985) *Nature 313*, 600–602.

Paetkau, V., Shaw, J., Elliott, J. F., Havek, C., Pohajdak, B., & Bleackley, R. C. (1987) in *Molecular Cloning and Analysis of Lymphokines* (Webb, D. R., & Goeddel, D. V., Eds.) pp 73–93, Academic Press, New York.

Quarless, Sh. A., & Heinrich, G. (1986) *BioTechniques 4*, 434–438.

Robb, R. J. (1982) *Immunobiology 161*, 21–50.

Singh, L., & Jones, K. W. (1984) *Nucleic Acids Res. 12*, 5627–5638.

Sood, A. K., Pereira, D., & Weissman, Sh. M. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 616–620.

Taniguchi, T., Matsui, H., Fujita, T., Takaoka, Ch., Kushima, N., Yoshimoto, R., & Hamuro, J. (1983) *Nature 302*, 305–310.

Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.

Von Heijne, G., Blomberg, C., & Liljenstrom, H. (1987) *J. Theor. Biol. 125*, 1–14.

Watson, J. D. (1970) *Molecular Biology of the Gene*, 2nd ed., W. A. Benjamin, New York.

Wegnez, M., Schachter, B. S., Baxter, J. D., & Martial, J. A. (1982) *DNA 1*, 145–153.

Williams, D. L., Newman, Th. C., Shelness, G. S., & Gordon, D. A. (1986) *Methods Enzymol. 128*, 671–689.