Because it would be useful for cell screening procedures to detect luciferase activity in undisrupted cultured cells, we tried to assay for the presence of luciferase activity in intact, living cells distributed in a microtiter plate. A cell line (B15-1) was derived from leaf protoplasts of the transgenic plant B15, and extracts from cells grown in suspension culture showed moderate levels of luciferase activity (210 LU per microgram of protein). Various numbers of cells were distributed into a microtiter dish, incubated in growth medium in the presence of different concentrations of luciferin and exposed for 1 hour to x-ray film (Kodak OG). Wells with luciferase-containing cells produced strong light signals (Fig. 6). After 1 hour of exposure, minimal signals were detected from 375 to 750 cells in 80 $\mu M$ luciferin. Cells survived the lower concentrations of luciferin (80 $\mu M$ or less) in long-term incubations (several weeks), but higher luciferin concentrations (400 $\mu M$ and above) were apparently toxic and blocked further growth of the cells. Hence, at lower luciferin concentrations, this assay constitutes a simple, nondisruptive procedure for screening reasonably large numbers of independent cell lines for luciferase activity.

The luciferase gene is an important new tool for studying gene expression in both plant and animal cells. Recently, de Wet *et al.* (*3*) demonstrated the expression of an SV40 promoter–driven luciferase gene in mammalian cells (monkey CV-1 cells). For plant genetic engineering purposes, the luciferase gene can be particularly valuable as a reporter of gene expression, as a marker in genetic crosses, and as a probe for a variety of plant cell functions. The speed and ease by which the luciferase assay can be performed permit more rapid screening of large numbers of transgenic plants or transformed cell lines. The luciferase gene is a sensitive reporter gene, because the luciferase assay itself can detect the light output from $3 \times 10^6$ molecules of luciferase (10 LU on a LKB model 1250 luminometer), which is at least 100 times more sensitive than a standard CAT assay (*3*). Furthermore, luciferase activity can be detected by simple x-ray film exposure or by more elaborate devices such as luminometers or image-intensifying video equipment. Luciferase offers many new possibilities as a reporter gene since detection of activity can be noninvasive and nondestructive, and luciferase activity can be assessed periodically over time, such as through the course of plant development. In addition, luciferase can be used as a cell marker to identify cells and as a tag to follow the targeting of proteins to various destinations within and outside of cells. Finally, because luciferase activity is influenced by a variety of parameters (*1*), light production from luciferase-expressing cells potentially could be used to monitor such events as ATP production and *p*H changes.


REFERENCES AND NOTES

1. M. DeLuca and W. D. McElroy, *Methods Enzymol.* **57**, 3 (1978).
2. J. R. de Wet, K. V. Wood, D. R. Helinski, M. DeLuca, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7870 (1985).
3. J. R. de Wet, K. V. Wood, M. DeLuca, D. R. Helinski, S. Subramani, in preparation.
4. J. Engebrecht, M. Simon, M. Silverman, *Science* **227**, 1345 (1985).
5. M. Fromm, L. P. Taylor, V. Walbot, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5824 (1985).
6. R. B. Horsch *et al.*, *Science* **227**, 1229 (1985).
7. R. N. Beachy *et al.*, *EMBO J.* **4**, 3047 (1985); L. Comai *et al.*, *Nature (London)* **317**, 741 (1985); G. K. Lamppa, G. Morelli, N.-H. Chua, *Mol. Cell. Biol.* **5**, 1370 (1985); G. K. Lamppa, F. Nagy, N.-H. Chua, *Nature (London)* **316**, 750 (1985); F. Nagy, G. Morelli, R. T. Fraley, S. G. Rogers, N.-H. Chua, *EMBO J.* **4**, 3063 (1985); C. Sengupta-Gopalan, N. A. Reichert, R. F. Barker, T. C. Hall, J. D. Kemp, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3320 (1985); J. Simpson *et al.*, *EMBO J.* **4**, 2723 (1985); R. Fluhr and N.-H. Chua, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2358 (1986); P. Powell *et al.*, *Science* **232**, 738 (1986); J. Simpson, M. Van Montagu, L. Herrera-Estrella, *ibid.* **233**, 34 (1986).
8. *Daucus carota* W001C suspension culture line from Z. Renee Sung.
9. The 3′ end fragment of the *nos* gene was obtained from pNCAT4 provided by J. Schell. The fragment extends from nucleotide position 1119 to 91 on the *nos* gene sequence from pTiT37, as described by M. Bevan, W. M. Barnes, and M.-D. Chilton [*Nucleic Acids Res.* **11**, 369 (1983)].
10. G. Heidecker and J. Messing, *Annu. Rev. Plant Physiol.* **37**, 439 (1986).
11. M. Bevan, *Nucleic Acids Res.* **12**, 8711 (1984).
12. K. V. Wood, J. R. de Wet, N. Dewji, M. DeLuca, *Biochem. Biophys. Res. Comm.* **124**, 592 (1985).
13. R. Fluhr, C. Kuhlemeier, F. Nagy, N.-H. Chua, *Science* **232**, 1106 (1986).
14. The plasmid pCaMV10 described by R. C. Gardner *et al.* [*Nucleic Acids Res.* **9**, 2871 (1981)] was provided by R. J. Shepherd.
15. S. N. Covey, G. P. Lomonossoff, R. Hull, *Nucleic Acids Res.* **9**, 6735 (1981); H. Guilley, R. K. Dudley, G. Jonard, E. Balazs, K. E. Richards, *Cell* **30**, 763 (1982).
16. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
17. T. Murashige and F. Skoog, *Physiol. Plant* **15**, 473 (1962).
18. M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).


19. We thank M. Fromm for advice on electroporation procedures, S. C. Lee for assistance in plant tissue culture, Z. Renee Sung for W001C cells, J. Schell for plasmid pNCAT4, R. J. Shepherd for plasmid pCaMV10, M. Bevan for the Ti plasmid binary vector Bin-19, and S. Rogers for *A. tumefaciens GV3111* containing the helper Ti-plasmids pTiT37SE and pTiB6S3SE. Supported by grant 85-CRCR-1-1540 from the U.S. Department of Agriculture Competitive Research Grants Program (to S.H.H.), grant PCM83-05446 from the National Science Foundation (to M.D.), by the University of California Biotechnology Research and Education Program, and by an NSF postdoctoral fellowship in plant biology (to D.W.O.).

22 August 1986; accepted 7 October 1986


# Novel Interleukin-2 Receptor Subunit Detected by Cross-Linking Under High-Affinity Conditions


MICHAEL SHARON, RICHARD D. KLAUSNER, BRYAN R. CULLEN, RICHARD CHIZZONITE, WARREN J. LEONARD



**Interleukin-2 (IL-2) binds to both high- and low-affinity classes of IL-2 receptors on activated T lymphocytes. Only the high-affinity receptors are involved in receptor-mediated endocytosis and normally transduce the mitogenic signals of IL-2; however, the structural features distinguishing the high- and low-affinity receptors are unknown. When $^{125}$I-labeled IL-2 was chemically cross-linked to activated human T lymphocytes, two major bands were identified. First, as predicted, a 68- to 72-kilodalton band, consisting of IL-2 (15.5 kilodaltons) cross-linked to the IL-2 receptor (55 kilodaltons), was observed. Second, an unpredicted 85- to 92-kilodalton moiety was detected. This band was not present when IL-2 was cross-linked to transfected C127 cells, which exclusively express low-affinity receptors. The data presented are most consistent with the existence of a 70- to 77-kilodalton glycoprotein subunit (p70) which, upon associating with the 55-kilodalton low-affinity receptor (p55), transforms it into a high-affinity site. It is proposed that p55 and p70 be referred to as the α and β subunits, respectively, of the high-affinity IL-2 receptor.**


THE LYMPHOKINE INTERLEUKIN-2 (IL-2) is synthesized and secreted by activated T cells and plays a critical role in the proliferative expansion of T-lymphocyte effector cells during the immune response (*1*). Concomitant with IL-2 production, two distinct classes of receptors with radically different affinities for IL-2 are expressed by activated lymphocytes (*2*). The ratio of low-affinity sites [with a reported dissociation constant ($K_d$) of 1 to 30 n$M$] to high-affinity sites (reported $K_d$ of 5 to 90 p$M$) is approximately 10:1 in both normal


M. Sharon, R. D. Klausner, W. J. Leonard, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
B. R. Cullen and R. Chizzonite, Department of Molecular Genetics, Hoffmann–La Roche, Inc., Nutley, NJ 07110.

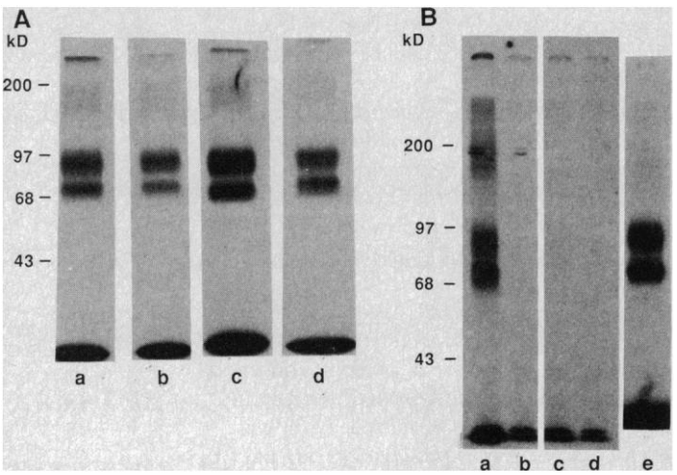


Fig. 1. Affinity labeling of cells with $^{125}$I-labeled IL-2. (A) DSS-mediated cross-linking of (lane a) PHA-activated lymphocytes, (lane b) PMA-activated lymphocytes, (lane c) HUT-102B2 cells, and (lane d) MT-2 cells. Detergent lysates of cross-linked cells were directly analyzed on SDS-PAGE. (B) DSS-mediated cross-linking of HUT-102 cells shown in lane a is inhibited by preincubation with (lane b) 1000-fold excess unlabeled IL-2, (lane c) excess anti-Tac, and (lane d) excess rabbit antiserum to the IL-2 receptor (R3134), but (lane e) not by excess 7G7B6. Membranes isolated from cross-linked cells were analyzed on SDS-PAGE. Normal peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation and cultured for 72 hours in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and either PHA (Burroughs Wellcome) at 50 μg/ml or PMA (Sigma) at 50 ng/ml. Cells were then harvested for cross-linking studies. HUT-102B2 cells and MT-2, both of which are infected with HTLV-I, were grown in RPMI 1640 supplemented with 10% FBS. $^{125}$I-labeled IL-2 preparations demonstrate binding affinities that are consistent with previously published values (*2, 3*) and yield identical results in cross-linking studies. The addition of a fixed amount of ligand to increasing numbers of cells indicated that 50% to 70% could be bound. The specific activity was calculated assuming that all $^{125}$I was associated with IL-2 and that only material that binds is bioactive. Cells were harvested and resuspended at $5 \times 10^6$ cells per milliliter in RPMI 1640 containing 25 m*M* Hepes and 1% BSA (Miles) at *p*H 7.4. In (B) (lanes b to e), cells were preincubated with recombinant IL-2 or antibodies to the receptor at 4°C for 1 hour. The binding was performed with the cells tumbling in the presence of $^{125}$I-labeled IL-2 for 1 hour at 4°C. Cells were pelleted at 400*g* and resuspended in 10 ml of phosphate-buffered saline, *p*H 8.3, containing 1 m*M* $MgCl_2$. Cross-linking was done according to modifications of the method of Brenner *et al.* (*17*). The cross-linker, DSS (Pierce Chemical), was dissolved at 10 mg/ml in DMSO and added to the cell suspensions to attain a final concentration of 20 to 100 μg/ml. Cells were tumbled at 4°C for 20 minutes and then a mixture of 10 m*M* tris and 1 m*M* EDTA, *p*H 7.4 (TE), was added to twice the volume. Cells were pelleted at 400*g* and then either extracted in a mixture of 300 m*M* NaCl, 50 m*M* tris, and 0.5% NP40, *p*H 7.4 (extraction buffer) (80 μl per $5 \times 10^6$ cells), or membranes were prepared by Dounce homogenizing in TE (1 ml per $6 \times 10^6$ cells), separating nuclei with a 400*g* centrifugation, and pelleting membranes at 100,000*g*. Results of cross-linking studies were identical whether portions of the postnuclear lysate (120 μl) or membrane pellets were boiled in SDS and analyzed on SDS-PAGE under reducing conditions.

activated lymphocytes and in most IL-2 receptor–expressing T-cell lines tested (*2, 3*). Current evidence points to the high-affinity receptors as the mediators of the biological activity of IL-2 (*2*). In addition, recently acquired data suggest that only IL-2 bound to high-affinity receptors is internalized via receptor-mediated endocytosis (*4*). The molecular basis for the distinction between these two classes of receptors remains unknown.

Characterization of the binding moiety for IL-2 on cells was facilitated by the development of a monoclonal antibody that reacts with activated T cells (anti-Tac) (*5*). This antibody suppresses IL-2–induced proliferation of T lymphocytes (*6*) and blocks both high- and low-affinity binding of radiolabeled IL-2 (*2, 6*). The Tac antigen has been characterized as a 55-kD glycoprotein that is capable of binding IL-2 (*7*). We refer to this protein as p55, acknowledging that apparent molecular sizes differ slightly among the different species and cell lines. The evidence clearly supports the notion that p55 is an IL-2 receptor–binding moiety on lymphocytes (*2, 6–8*).

Complementary DNA's (cDNA's) of both the human and mouse p55 have been cloned and expressed (*9*). Stable transformants of mouse L cells transfected with the human cDNA express only low-affinity binding (*10*). Two groups have demonstrated that, unlike L cells, T lymphocytes express both high- and low-affinity receptors when transfected with a human p55 cDNA (*11*). In addition, fusion of membranes derived from L-cell transfectants expressing only low-affinity murine IL-2 receptors with membranes derived from activated human T cells appears to result in partial conversion of low-affinity murine receptors to the high-affinity state (*12*). These data suggest that undefined components expressed in T-lymphocyte membranes but not in fibroblasts are capable of interacting with the IL-2–binding moiety, p55, to reconstitute the high-affinity IL-2 receptor.

Affinity cross-linking has been used to investigate the interaction of various peptide hormones and their receptors (*13*). We used the homobifunctional reagent disuccinimidyl suberate (DSS) to detect membrane components that interact with IL-2. $^{125}$I-labeled IL-2 was bound under high-affinity conditions (100 p*M*) to normal human peripheral blood lymphocytes after activation with phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) and to the HTLV-I–infected, adult T-cell leukemia (ATL)–derived cell lines HUT-102B2 and MT-2. The HTLV-I–infected cell lines express five to ten times as many IL-2 binding sites as lymphoblasts do (*2, 3*). Disuccinimidyl suberate was used to covalently cross-link $^{125}$I-labeled IL-2 to the surface of intact cells. Detergent lysates of treated cells were prepared and directly analyzed by SDS–polyacrylamide electrophoresis (SDS-PAGE) (Fig. 1A). Three broad bands are visible in the autoradiograph in addition to the IL-2, which migrates at 15.5 kD. The lowest band migrates at 68 to 72 kD in HUT-102B2 cells, MT-2 cells, and lymphoblasts. We expected to see this band since it corresponds to the sum of the molecular sizes of IL-2 (15.5 kD) and p55 (the IL-2–binding moiety) (*7*). An unexpected and similarly intense second band with an apparent molecular size of 85 to 92 kD was detected in both ATL-derived cell lines and lymphoblasts. A third, less intense band, broadly spanning 160 to 200 kD, was quite

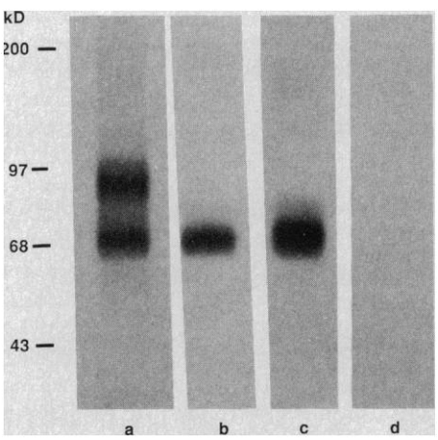


Fig. 2. Immunoprecipitation of affinity-labeled HUT-102B2 cell lysates. Cells cross-linked with $^{125}$I-labeled IL-2 were extracted as described in the legend to Fig. 1, and 400 μl of lysate was subjected to immunoprecipitation either with (lane a) a monoclonal antibody to IL-2 (17A1), (lane b) a monoclonal antibody to the IL-2 receptor (7G7B6), (lane c) a polyclonal antibody to the IL-2 receptor (R3134), or (lane d) an antiserum to bovine albumin. The antibodies were adsorbed to protein A Sepharose beads (Pharmacia). After being washed once in 1 ml of extraction buffer, the beads were combined with lysate at 0.65% SDS for 20 minutes at room temperature, then washed extensively before being boiled in SDS and subjected to electrophoresis under reducing conditions.

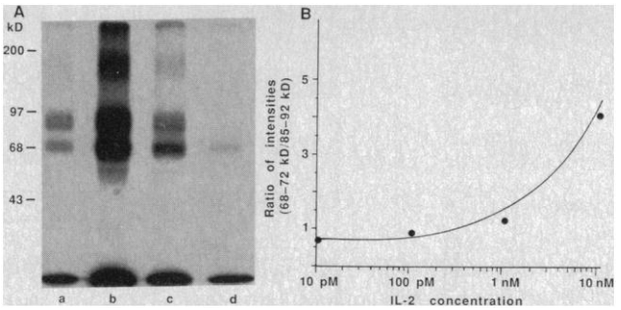


Fig. 3. Affinity labeling of HUT-102B2 cells at a range of IL-2 concentrations was performed as described in the legend to Fig. 1. (A) $^{125}$I-labeled IL-2 was bound at (lane a) 10 p*M*, (lane b) 100 p*M*, (lane c) 1.0 n*M*, and (lane d) 10.0 n*M* before DSS was added. The specific activities of ligand added in lanes c and d were diluted 10-fold and 100-fold, respectively, over that used in lanes a and b with unlabeled IL-2. (B) The ratio of intensities of the 68- to 72-kD band to those of the 85- to 92-kD band obtained by densitometric analysis of lanes in (A) plotted against IL-2 concentration. Intensities were adjusted appropriately for dilutions in specific activity.

variable from experiment to experiment and its exact identity remains unknown.

We next demonstrated that these bands arose from the specific binding and cross-linking of IL-2 to IL-2 receptors (Fig. 1B). HUT-102B2 cells were incubated for 1 hour at 4°C with excess unlabeled IL-2 or with various antibodies directed against p55 before binding and cross-linking to labeled IL-2. Incubation of cells with excess unlabeled IL-2 blocked the appearance of all cross-linked species (lane b). Two antibodies that block specific IL-2 binding, anti-Tac, a monoclonal antibody, and R3134, a polyclonal antiserum (*6, 14*), also blocked the production of specific cross-linked species (lanes c and d). In contrast, incubation with 7G7B6, a monoclonal antibody to p55 recognizing a site distinct from the IL-2–binding region (*15*), did not block the appearance of either the 68- to 72-kD or the 85- to 92-kD bands (lane e). Neither cross-linking of labeled IL-2 to unstimulated Jurkat cells, which express no detectable IL-2 receptors (*2*), nor sham treatment of labeled IL-2 bound to HUT cells (omitting DSS) resulted in the appearance of the 68- to 72-kD and the 85- to 92-kD bands.

These experiments indicated that cross-linking of $^{125}$I-labeled IL-2 into both the 68- to 72-kD and 85- to 92-kD species was blocked by antisera directed against the IL-2 binding domain of p55. In order to further characterize the components of these bands, we carried out immunoprecipitations with a monoclonal antibody (17A1) directed against IL-2 and monoclonal (7G7B6) and polyclonal (R3134) antibodies directed against p55 (Fig. 2). Precipitations with 17A1 (lane a) performed on lysates of cross-linked HUT-102B2 cells identified both the 68- to 72-kD and 85- to 92-kD bands, thereby demonstrating that both species represent material cross-linked to IL-2. Immunoprecipitations performed under identical conditions with antibodies 7G7B6 or R3134 (lanes b and c), both directed against p55, recognized only the 68- to 72-kD species. These two antibodies together recognize a minimum of three independent epitopes of p55 (*14, 15*). Immunoprecipitation with R3134 (lane c) was performed on affinity-labeled lysates that were first boiled in SDS to denature the complexes and possibly expose additional determinants not present in the native state. Identical results were obtained when lysates were not boiled before immunoprecipitation. No 85- to 92-kD complex was immunoprecipitated with antibodies to p55 under various conditions that readily allowed identification of the lower band. We have been unable to immunoprecipitate any complexes from affinity-labeled lysates by using a rabbit antibody to bovine serum albumin (BSA). This result rules out the possibility that the 85- to 92-kD band results from the cross-linking of $^{125}$I-labeled IL-2 to BSA carried over from our growth media or binding buffer (lane d).

We next further investigated the relation between the 85- to 92-kD complex and high-affinity binding. Labeled IL-2 was cross-linked to HUT-102 cells at increasing IL-2 concentrations spanning the $K_d$'s of the high- and low-affinity sites (Fig. 3). Affinity labeling under high-affinity conditions generates the 68- to 72-kD and 85- to 92-kD bands. Although there is no a priori reason to predict it, these bands are of similar intensity. As IL-2 concentration approaches low-affinity conditions, the relative prevalence of these two bands changes (Fig. 3A). The ratios of the densitometric intensities of the lower to upper bands were plotted as a function of IL-2 concentration (Fig. 3B). As the IL-2 concentration approached the $K_d$ of the low-affinity IL-2 receptor a 4:1 ratio of intensities of lower to upper bands was observed. This is consistent with a known tenfold greater number of low-affinity sites than of high-affinity sites. These results are compatible with the hypothesis that components of both complexes are required jointly for binding under high-affinity conditions, but only p55 is involved in low-affinity binding.

To test this hypothesis, we cross-linked $^{125}$I-labeled IL-2 to 21N5, a cell line generated by transfecting murine C127 epithelial cells with a bovine papilloma virus (BPV) expression vector construct containing the human p55 gene. These cells express $0.5 \times 10^6$ to $1.5 \times 10^6$ receptors per cell, all of which are low-affinity sites. The cross-linked 21N5 cells display only a 68- to 72-

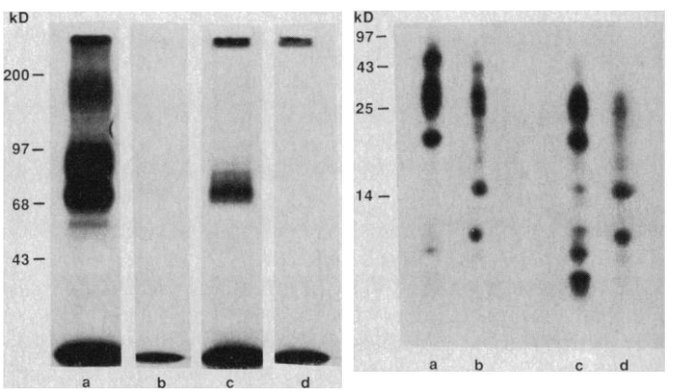


Fig. 4 (left). Affinity labeling of (lane a) HUT-102B2, (lanes b and c) 21N5, and (lane d) ID13 cells. $^{125}$I-labeled IL-2 was bound at (lanes a and b) 100 p*M* and (lanes c and d) 1.0 n*M* before DSS was added. 21N5 cells were prepared by one of us (B.R.C.) and represent murine C127 cells transformed with a BPV expression vector (*18*) containing the 879–base pair Nci I fragment of pIL2R3 (*9*). 21N5 cells contain more than 100 copies of the IL-2 receptor gene per cell. ID13 is an established, C127-derived BPV-1–transformed cell line (*19*). Both 21N5 and ID13 cell lines were grown in Dulbecco's minimum essential medium with 10% FBS and harvested for cross-linking studies by scraping. Fig. 5 (right). Peptide analysis of (lanes b and d) 68- to 72-kD, and (lanes a and c) 85- to 92-kD bands with V-8 protease used at concentrations of 10 μg/ml in lanes a and b and 100 μg/ml in lanes c and d. $^{125}$I-labeled IL-2 was bound and cross-linked to HUT-102B2 cells with DSS as described in the legend to Fig. 1. Cell lysates were immunoprecipitated with the 17A1 monoclonal antibody as described in the legend to Fig. 2. Immunoprecipitates were subjected to SDS-PAGE on a 7.5% gel and exposed, unfixed, to Kodak film. Bands of interest were excised and loaded into wells of a 15% SDS gel and overlayed with V-8 protease in a mixture of 0.1*M* tris, 20% glycerol, 0.1% SDS, and 0.1% bromphenol blue (*p*H 6.8). Samples were advanced by electrophoresis into the stacking gel and left there for 40 to 50 minutes with the power turned off (*20*). Electrophoresis was then completed in the usual fashion, and autoradiograms were prepared.

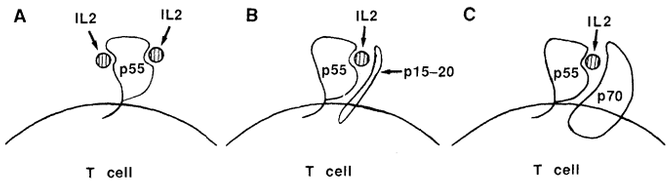


Fig. 6. Models of the high-affinity IL-2 receptor.

kD complex at both 100 p*M* (lane b) and 1.0 n*M* (lane c) IL-2 (Fig. 4). HUT-102B2 cells, which express both high- and low-affinity sites, were cross-linked in parallel and display both the 85- to 92-kD and the 68- to 72-kD bands at 100 p*M* IL-2 (lane a). Neither band was detected in cross-linking experiments with C127 cells transfected with a BPV expression vector that did not contain the p55 gene (lane d). The absence of an upper band in affinity-labeled 21N5 cells is consistent with the hypothesis that a component of the 85- to 92-kD complex is correlated with high-affinity binding.

The inability to immunoprecipitate the 85- to 92-kD complex with any anti-p55 reagent suggested that p55 is not a component of this moiety. To further differentiate the 68- to 72-kD from the 85- to 92-kD cross-linked complexes, we subjected each to partial proteolysis with V8 protease and analyzed the fragments by SDS-PAGE. The results demonstrate that different peptide maps are derived from the two complexes (Fig. 5). Qualitatively different peptide patterns were also found when partial chemical cleavage was performed with cyanogen bromide (*14*). This finding supports the conclusion from the immunoprecipitation data that the 68- to 72-kD complex is not part of the 85- to 92-kD complex. Preliminary experiments involving glycosidase treatment of the 85- to 92-kD band suggests that the uncharacterized protein moiety complexed with IL-2 is a glycoprotein.

We have shown that affinity labeling of the IL-2 binding region identifies two different species. The prevalence of these species depends on the concentration of ligand in a manner that allows us to correlate the 85- to 92-kD complex with high-affinity binding, whereas the 68- to 72-kD complex is correlated with all binding conditions. The absence of the 85- to 92-kD moiety in a transformant expressing large numbers of exclusively low-affinity binding sites suggests a direct role for a component of this complex as part of the high-affinity receptor.

Proteins of 113 kD and 180 kD have been previously identified in anti-Tac immunoprecipitations of lymphoblasts and HUT-102B2 cells metabolically labeled with [$^{35}$S]methionine (*6, 7*). Both of these proteins are too large to account for the 85- to 92-kD moiety; however, it is conceivable that one or both of these represent other subunits associated with IL-2 receptors.

What then are the components of these two cross-linked species? Both contain IL-2, as demonstrated by (i) the recognition of both with a monoclonal antibody directed against IL-2 and (ii) the observation that identical 15.5-kD molecules are released from both species when cleavable cross-linking reagents are used (*14*). The ability to immunoprecipitate specifically the 68- to 72-kD species with monoclonal or polyclonal antibodies to p55 establishes that this complex can be explained as a covalent heterodimer of IL-2 and p55. The complete nature of the 85- to 92-kD complex is less apparent. An unlikely possibility is that there are entirely separate binding moieties for the high- and low-affinity receptors and that the 85- to 92-kD entity represents IL-2 cross-linked to a binding site on a species distinct from p55. The evidence of epitopes shared by high- and low-affinity sites in the human (both bind anti-Tac, 7G7B6, and R3134) and mouse (both bind monoclonal antibodies 7D4 and 3C7) (*16*), as well as the aforementioned reconstitution studies (*11, 12*), make this model untenable.

We can consider three models for the high-affinity IL-2 receptor to explain the 85- to 92-kD complex (Fig. 6). In the first two models (Fig. 6, A and B) the 85- to 92-kD moiety represents a ternary complex in which a 15- to 20-kD entity is additionally cross-linked to the 68- to 72-kD complex. This 15- to 20-kD entity could either be a second IL-2 molecule or a yet unidentified, associated 15- to 20-kD receptor subunit. There are several arguments against each of these two models. First, the inability to precipitate the 85- to 92-kD complex with any anti-p55 reagent suggests that p55 is not part of this species. The different peptide maps obtained from the 68- to 72-kD and 85- to 92-kD bands are consistent with this conclusion. Second, chemical cross-linking is generally a very inefficient process. The prospect of IL-2, under high-affinity binding conditions, being equivalently cross-linked in a bimolecular complex (IL-2 plus p55) and a trimolecular complex (IL-2 plus p55 plus a 15- to 20-kD entity) seems unlikely. In addition, our cross-linking data from a transformant expressing exclusively low-affinity binding greatly diminishes the possibility that each p55 is capable of binding two IL-2 molecules. Cross-linking to solubilized, affinity-purified p55 obtained from HUT-102B2 cells confirms that only one IL-2 binds to most p55 molecules (*14*). For model A to be consistent with these data, p55 would have to undergo some T cell–specific modifications that allow it to bind two IL-2 molecules only under high-affinity conditions. The recent work of Robb (*12*) wherein T-cell membranes "donate" a factor, unable to bind IL-2, that converts low-affinity receptors to high-affinity sites argues against such modifications.

We believe the most likely model is portrayed in Fig. 6C. In this model, the high-affinity IL-2 receptor results from the noncovalent interaction of p55 with a closely associated 70- to 77-kD membrane protein that we denote p70. IL-2 binds to p55 under high-affinity conditions but also interacts with p70. This enables the cross-linker to link IL-2 with either p55 (68- to 72-kD complex) or p70 (85- to 92-kD complex). It is unclear whether or not p70 is independently capable of binding IL-2 directly. We know that the 70-kD protein must be physically close enough to bound IL-2 (within 11 Å) to be cross-linked to it by DSS. The appearance of this 85- to 92-kD complex is completely blocked by anti-Tac and R3134 antibodies (Fig. 1B), both of which bind to p55 and not to p70. If p70 can bind IL-2 directly, the presence of the antibodies on the IL-2 binding region of p55 must be capable of sterically interfering with the interaction between IL-2 and p70. Considering the proximity of p70 to p55 it is possible that p70 directly contributes to the binding and that together p70 and p55 establish the high-affinity interaction. Alternatively, if p70 cannot bind IL-2, an allosteric interaction between p70 and p55 may raise the affinity of the p55 binding site for IL-2. In either case, the low-affinity IL-2 receptor would be explained by a p55 molecule that is not associated with p70. We propose that p55 and p70 be referred to as the α and β subunits, respectively, of the high-affinity IL-2 receptor.

It is generally assumed that the cytoplasmic domains of receptors mediate endocytosis. The p70 subunit model may provide a mechanism whereby occupied high-affinity sites would be selectively internalized in spite of the short cytoplasmic domain of p55 (*4, 9*). Although the exact role of receptor-mediated endocytosis in signal transduction is unclear, the known correlation with both high-affinity binding and biological function in this system is striking. Identification of one or more uncharacterized components of the 85- to 92-kD complex may elucidate the relation of these processes to signal transduction.

*Note added in proof*: Since submission of this manuscript, we have directly identified p70. IL-2 was cross-linked to [$^3$H]mannose-labeled HUT-102B2 cells with a cleavable cross-linker, and 17A1 immunoprecipitates were subjected to electrophoresis on a two-dimensional nonreducing-reducing gel system. p70 was identified as a 65- to 77-kD doublet below the diagonal consistent with cleavage of a single IL-2 molecule.


REFERENCES AND NOTES

1. K. A. Smith, *Immunol. Rev.* **51**, 337 (1980).
2. R. J. Robb, A. Munck, K. A. Smith, *J. Exp. Med.* **154**, 1455 (1981); R. J. Robb, W. C. Greene, C. M. Rusk, *ibid.* **160**, 1126 (1984).
3. J. W. Lowenthal, R. H. Zubler, M. Nabholz, H. R. MacDonald, *Nature (London)* **315**, 669 (1985); T. Uchiyama *et al.*, *J. Clin. Invest.* 76, 446 (1985).
4. A. M. Weissman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1463 (1986); M. Fujii *et al.*, *J. Exp. Med.* **163**, 550 (1986).
5. T. Uchiyama, S. Broder, T. A. Waldmann, *J. Immunol.* **126**, 1393 (1981).
6. W. J. Leonard *et al.*, *Nature (London)* **300**, 267 (1982).
7. W. J. Leonard, J. M. Depper, R. J. Robb, T. A. Waldmann, W. C. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6957 (1983); W. J. Leonard *et al.*, *J. Biol. Chem.* **260**, 1872 (1985).
8. R. J. Robb and W. C. Greene, *J. Exp. Med.* **158**, 1332 (1983).
9. W. J. Leonard *et al.*, *Nature (London)* **311**, 626 (1984); T. Nikaido *et al.*, *ibid.*, p. 631; D. Cosman *et al.*, *ibid.* **312**, 768 (1984); J. Miller *et al.*, *J. Immunol.* **134**, 4212 (1985).
10. W. C. Greene *et al.*, *J. Exp. Med.* **162**, 363 (1985).
11. M. Hatakeyama *et al.*, *Nature (London)* **318**, 467 (1985); S. Kondo *et al.*, *ibid.* **320**, 75 (1986).
12. R. J. Robb, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3992 (1986).
13. P. F. Pilch and M. P. Czech, in *Molecular and Chemical Characterization of Membrane Receptors*, C. Venter *et al.*, Eds. (Liss, New York, 1984), pp. 161–175.
14. M. Sharon and W. J. Leonard, unpublished observations.
15. L. A. Rubin *et al.*, *Hybridoma* **4**, 91 (1985).
16. G. Ortega, R. J. Robb, E. M. Shevach, T. R. Malek, *J. Immunol.* **133**, 1970 (1984).
17. M. B. Brenner, I. S. Trowbridge, J. L. Strominger, *Cell* **40**, 183 (1985).
18. G. N. Pavlakis and D. H. Hamer, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 397 (1983).
19. L. P. Turek *et al.*, *ibid.* **79**, 7914 (1982).
20. D. W. Cleveland, S. G. Fischer, M. W. Kirschner, U. K. Laemmli, *J. Biol. Chem.* **252**, 1102 (1977).


21. We thank J. Harford and A. Weissman, NIH, for valuable discussions, K. Perry for her excellent secretarial assistance, M. Popovic, NCI, for HUT-102B2 cells, F. Ruscetti, NCI, for MT-2 cells, P. Howley, NCI, for ID13 cells, T. A. Waldmann, NCI, for anti-Tac, and W. E. Biddison, NIH, and D. Nelson, NCI, for 7G7B6. Rabbit antiserum to bovine albumin was obtained from Cooper Biomedical, $^{125}$I-labeled IL-2 was initially provided by P. Kilian, Hoffmann–La Roche, and later obtained from New England Nuclear. Unlabeled IL-2 was a gift of F. Khan, P. Kilian, and J. Jensen, Hoffmann–La Roche.

1 July 1986; accepted 23 September 1986


# Curvilinear, Three-Dimensional Motion of Chromatin Domains and Nucleoli in Neuronal Interphase Nuclei


UMBERTO DE BONI AND ARLAN H. MINTZ



**The term "nuclear rotation" refers to a motion of nucleoli within interphase nuclei of several cell types. No mechanism or function has been ascribed to this phenomenon, and it was unknown whether nuclear structures in addition to nucleoli participate in this motion. Moreover, it was unclear whether nuclear rotation occurs independent of concurrent motion of juxtanuclear cytoplasm. The work reported here presents quantitative evidence, for three-dimensional intranuclear, tandem motion of fluorescently labeled chromatin domains associated with nucleoli and those remote from nucleoli. The results show that such motion is curvilinear, that it is not restricted to nucleoli, and, moreover, that it occurs independently of motion of juxtanuclear, cytoplasmic structures. These results suggest that this motion represents karyoplasmic streaming and its function is to transpose to nuclear pores those chromatin domains actively transcribed.**


MOTION OF NUCLEOLI IN INTERphase nuclei has traditionally been termed nuclear rotation (NR) (*1–6*). Initial, descriptive observations of rotary movements of nucleoli in nuclei of neurons (*7, 8*) were followed by quantitative analyses of NR in neurons by Pomerat *et al.* (*9*) and others (*10*). In nonneuronal cells, NR has been documented for human olfactory epithelial cells (*11*) and for several other cell types (*5, 12–15*). Results showed that rates of NR in cultured cells, defined by displacement of the nucleolus, range from 1 revolution per minute in olfactory epithelial cells to 1 revolution per hour and slower rates in cultured neurons. No mechanism or functional significance has been assigned to this phenomenon. Furthermore, it was not known whether subnuclear structures other than nucleoli participate in the observed motion and whether NR occurs independently of concurrent cytoplasmic movement.

We present here evidence for motion, in three dimensions, of DAPI (4,6-diamidino-2-phenylindole)–stained, fluorescent chromatin domains in tandem with nucleoli (Fig. 1), in interphase nuclei of dorsal root neurons (DRN) in vitro. Further, we present evidence that such motion occurs independently of concurrent motion of cytoplasmic structures, including those in close proximity to the outer nuclear membrane.

Linear displacements of nucleoli in DRN, derived from measurements of displacement of phase-dark nucleoli and analyzed in a two-dimensional (2-D) plane, occur at mean rates of 0.077 ± 0.003 μm/min (± SEM; $n$ = 269; range, 0 to 0.39 μm/min), which correspond to mean apparent angles subtended at the center of spherical nuclei of 0.746 ± 0.03 deg/min (with a range of 0 to 4.8 deg/min).

Observation of NR on time-lapse cine film shows that phase-dense granules, distributed throughout the nucleus, clearly move in tandem with nucleoli. To verify that these granules represent nuclear domains, and to quantify their motion, we used DAPI to vitally stain nuclei. We measured the location of intranuclear domains in seven neuronal nuclei, typically at intervals of 15 to 30 minutes, over periods from 120 to 420 minutes. The dynamics of nucleoli and DAPI-stained chromatin domains were essentially identical. For example, in the nucleus shown in Fig. 1, the nucleolus, one

Table 1. Comparison among representative translocation rates (T) and rotation rates (R) of nucleoli and of DAPI-stained fluorescent, nucleolus-associated and nonnucleolus-associated chromatin domains. Note absence of significant differences in rates (ANOVA, $P > 0.05$). Means ± SEM; $n$ = 6.

| Nucleolus | | DAPI-stained domains | | | |
|---|---|---|---|---|---|
| | | Nucleolus associated | | Nonnucleolus associated | |
| T (μm/min) | R (deg/min) | T (μm/min) | R (deg/min) | T (μm/min) | R (deg/min) |
| | | *2-D planar assay* | | | |
| 0.065 ± 0.01 | 0.608 ± 0.12 | 0.097 ± 0.03 | 0.823 ± 0.31 | 0.093 ± 0.01 | 0.910 ± 0.13 |
| | | *3-D dot-product assay* | | | |
| 0.093 ± 0.01 | 1.632 ± 0.27 | 0.070 ± 0.01 | 1.872 ± 0.30 | 0.070 ± 0.01 | 1.825 ± 0.16 |


Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.