Npys group was removed, and Npys-Ala was coupled. The Boc group was removed and the Ac-His chain was assembled (Boc chemistry). The Npys group was removed and the Ac-Glu chain was assembled (Boc chemistry). Every coupling reaction was monitored for completeness and repeated if necessary. Ac, acetyl; Boc, *tert*-butyloxycarbonyl; Fmoc, 9-fluorenylmethoxycarbonyl; Npys, 3-nitro-2-pyridylsulfenyl.

15. J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis* (Pierce Chemical, Rockford, IL, 1984).
16. R. Matsueda and R. Walter, *Int. J. Peptide Protein Res.* **16**, 392 (1980).
17. I. V. Berezin, N. F. Kazanskaya, A. A. Klyosov, *FEBS Lett.* **15**, 121 (1971).

18. Supported by Office of Naval Research contract N00014-86-K-0476 and UCHSC BRS grant 888. We thank J. R. Cann and R. Coombs for the CD measurements and R. Binard for amino acid analyses.

6 February 1990; accepted 19 April 1990

β-Arrestin: A Protein That Regulates β-Adrenergic Receptor Function

MARTIN J. LOHSE, JEFFREY L. BENOVIC,* JUAN CODINA, MARC G. CARON, ROBERT J. LEFKOWITZ

Homologous or agonist-specific desensitization of β-adrenergic receptors is thought to be mediated by a specific kinase, the β-adrenergic receptor kinase (βARK). However, recent data suggest that a cofactor is required for this kinase to inhibit receptor function. The complementary DNA for such a cofactor was cloned and found to encode a 418–amino acid protein homologous to the retinal protein arrestin. The protein, termed β-arrestin, was expressed and partially purified. It inhibited the signaling function of βARK-phosphorylated β-adrenergic receptors by more than 75 percent, but not that of rhodopsin. It is proposed that β-arrestin in concert with βARK effects homologous desensitization of β-adrenergic receptors.

HOMOLOGOUS DESENSITIZATION is a widespread process that causes specific dampening of cellular responses to stimuli such as hormones, neurotransmitters, or sensory signals (*1*). It is defined by a loss of responsiveness of receptors that have been continuously or repeatedly stimulated, while responses of other receptors remain intact. The β-adrenergic receptor–G_s–adenylyl cyclase system has been studied as a model system for this phenomenon (*1*). Homologous desensitization of β receptors has been shown to be associated with phosphorylation of the receptors, which appears to be catalyzed by a specific kinase, called β-adrenergic receptor kinase (βARK) (*2*). This kinase phosphorylates only agonist-occupied receptors, which may explain the receptor specificity of homologous desensitization. βARK-mediated phosphorylation has been shown for several G protein–coupled receptors. Several lines of evidence indicate that βARK participates in homologous desensitization of β-adrenergic receptors (*3*). The recent cloning of the cDNA encoding βARK suggests that there are several isoforms of this enzyme whose specificities have not yet been determined (*4*).

The phosphorylation of purified reconstituted β_2 receptors by crude βARK preparations causes significant impairment of their capacity to activate G_s (*5*), the guanine nucleotide binding protein that serves as a signal transducer for β receptors. However, this inhibitory effect is almost completely lost when βARK is purified, although the pure preparations retain the phosphorylating activity (*5*). These data suggest that a cofactor (or more) is necessary to effect βARK-mediated inhibition of receptor function, and that this cofactor is lost during the purification of βARK.

The existence of such cofactors is further suggested by analogies with the light-activated rhodopsin-phosphodiesterase system in the retina. Phosphorylation of light-activated rhodopsin by rhodopsin kinase requires the binding of an additional protein, termed arrestin, to inhibit rhodopsin function (*6*). This analogy is supported by the observation that arrestin enhances the inhibitory effects of βARK-mediated phosphorylation on β-receptor function, as assessed in a reconstituted system with the pure components (*5*). However, large amounts of arrestin are required for a relatively modest inhibition, indicating that arrestin itself is not the protein that serves to inhibit β-receptor function. These observations suggested that there might be one (or more) arrestin-like protein that inactivates βARK-phosphorylated β receptors, and possibly other G protein–coupled receptors.

The search for arrestin-like cDNA's was performed with a 1262-bp probe comprising the entire coding sequence of arrestin (*7*). Screening of a bovine brain library revealed positive clones at a frequency of 1 in 10,000 at relatively high stringency (0.5× SSC at 65°C), eight of which were isolated and sequenced. All contained the full-length or part of the same open reading frame (ORF) of

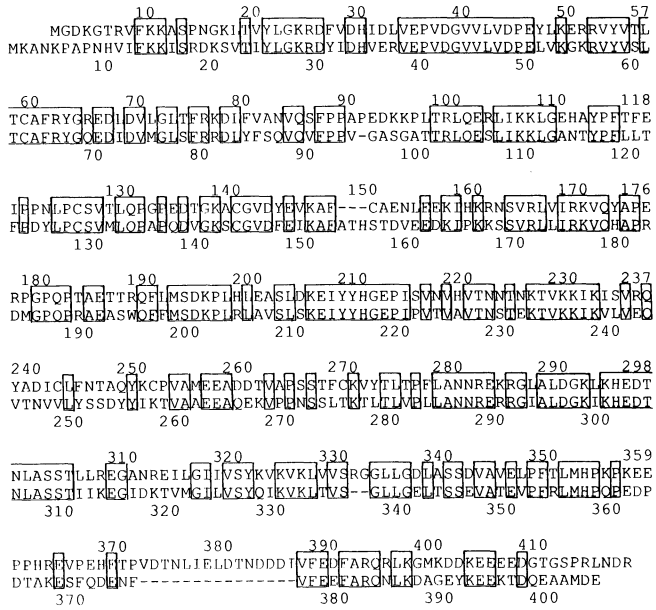

Fig. 1. Deduced amino acid sequence of β-arrestin (top sequence) and arrestin (bottom sequence). Gaps were introduced to obtain maximum similarity. Boxes surround identical residues. The sequence of arrestin was taken from Shinohara *et al.* (*7*). A randomly primed, size-selected (2 to 4.4 kb) bovine brain library in λZAP with 300,000 independent clones (*20*) was screened with a 1262-bp Hpa I–Nhe I fragment of bovine arrestin cDNA (*7*, *20*) containing the entire coding sequence of arrestin. Eight positive clones were isolated, sequenced in both directions (dideoxynucleotide method), and found to contain all or parts of the same 1254-bp open reading frame. The 3′ ends of the clones varied from 0.6 to 2.5 kb, but none contained a poly(A) tail. The complete nucleotide sequence is deposited in GenBank (accession number M33601) or is available from the authors.

M. J. Lohse, J. L. Benovic, M. G. Caron, R. J. Lefkowitz, Howard Hughes Medical Institute, Departments of Medicine, Biochemistry and Cell Biology, Durham, NC 27710.
J. Codina, Department of Cell Biology, Baylor College, Houston, TX.

*Present address: Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140.

Table 1. Similarities between bovine β-arrestin and the alpha subunits of different G proteins from rat. The predicted sequences of the G protein alpha subunits and their alignment are taken from Jones and Reed (*14*). Identical amino acids or conservative substitutions are printed in boldface, with the following conservative categories: C; S,T,P,A,G; N,D,E,Q; H,R,K; M,I,L,V; and F,Y,W.

Peptide	Region				
	1	2	3	4	5
β-arrestin	74 **TF**RK**DL**	94 **KK-PLT**	291 **GK**LK**HED**	379 **LDTNDDDIVF**	402 **DK**EEEE**DG**
$G\alpha_s$	213 Q**V**-D**KV**	304 G**K**SK**IE**	304 **GK**SK**IED**	361 **VDTENIRRVF**	27 **EKNLKEDG**
$G\alpha_o$	191 **TF-KNL**	280 **KKSPLT**	280 KKSPLTI	327 TDTNNIQVVF	20 **EKNLKEDG**
$G\alpha_{i2}$	191 **TF-KDL**	280 TQS**PLT**	280 TQSPLTI	328 TDTKNVQFVF	20 **DKNLREDG**

1254 bp starting with an ACCATGG Kozak consensus sequence (*8*) for initiation of translation. Initiation of translation at this position was verified by in vitro translation; the coding region of the β-arrestin cDNA was cloned into pGEM-5Zf (Promega), and RNA was made and translated in a rabbit reticulocyte system (Promega), giving rise to a single major protein (48 kD).

The deduced amino acid sequence of the putative protein β-arrestin (Fig. 1), was compared with that of arrestin. The ORF codes for a 47.1-kD protein; this protein is slightly larger than arrestin (45.3 kD). There is a high degree of similarity with arrestin over the entire length of the protein, with an overall identity of 59 percent. Accounting for conservative substitutions, the degree of similarity is 75 percent. The most divergence occurs in the carboxyl-terminal region, where β-arrestin contains an additional stretch of 15 amino acids, and the amino and carboxyl termini themselves. Both proteins are hydrophilic, and the calculated isoelectric points of both proteins are similar (6.03 for arrestin, 5.84 for β-arrestin).

A search for similarities between β-arrestin and the α subunits of several G proteins (Table 1) revealed five stretches that showed homologies with the various α subunits, which may be of interest for two reasons. First, the α-subunit sequences of regions 3 and 4 represent carboxyl-terminal sequences that may be important for coupling of the α subunits to receptors (*9*). These homologies might allow β-arrestin to mimic G proteins and bind to receptors. Second, the sequence GKLKHED (column 3) has a counterpart only in α_s, possibly indicating that β-arrestin might interact preferentially with G_s-coupled receptors.

An expression vector for β-arrestin was constructed by inserting a Not I–Apa I fragment of the cDNA containing the coding region into the vector pBC12/CMV/IL-2 (*10*). The resulting vector, pBCβ-arrestin, was transfected into COS-7 cells by the DEAE-dextran procedure, and the cell cytosol preparations were tested for activity after 48 to 72 hours. As a control we prepared an analogous expression vector for arrestin by cloning the Hpa I–Nhe I fragment of the arrestin cDNA (*7*) into the same vector to create pBCarrestin. The cytosol preparations were tested for β-arrestin activity, that is, inhibition of the function of βARK-phosphorylated β_2-adrenergic receptors, by measuring the ability of the purified receptors to stimulate purified G_s in a reconstituted system (*5*).

When added in appropriate amounts, the cytosol preparations of β-arrestin–transfected cells did indeed inhibit the function of βARK-phosphorylated β_2 receptors (Table 2). This inhibition amounted to 30 to 40 percent (but twice as much inhibition was observed with purified β-arrestin as detailed below). No inhibition was seen with nonphosphorylated receptors. Cytosol preparations from cells transfected with pBC12 alone or with pBCarrestin had no inhibitory effect on either control or βARK-phosphorylated receptors. This suggests that, although arrestin in high concentrations can inhibit β-receptor function (*5*), β-arrestin is more potent in this respect.

Receptor specificity of β-arrestin and arrestin was determined from their effects on rhodopsin function (Table 2). To this end, the cytosol preparations were tested for their ability to inhibit the light-induced guanosine triphosphatase (GTPase) of purified transducin added to rod outer segments under phosphorylating conditions (*6*). In this system, only the cytosol preparation from arrestin-transfected cells inhibited the light-induced GTPase activity. These results indicate that, while arrestin is specific for rhodopsin compared to β receptors, β-arrestin preferentially inhibits β receptors.

β-Arrestin was partially purified from the cytosol of transfected COS-7 cells by sequential ion-exchange chromatography and gel filtration. On a Mono-Q anion-exchange column β-arrestin eluted at a salt concentration of ~230 mM, as assessed by the ability of the eluted fractions to inhibit the function of βARK-phosphorylated β_2 receptors. Arrestin eluted at only slightly lower salt concentrations (~200 mM). β-Arrestin contained in the peak fractions from the Mono-Q chromatography eluted on a Superose-12 gel filtration column with an estimated molecular mass of ~46,000. This partially purified β-arrestin inhibited the function of

Table 2. Specific inhibition of β_2-receptor function by expressed β-arrestin. Effects of the cytosol preparations of transfected COS-7 cells on β_2-receptor–mediated activation of G_s and on transducin activation by phosphorylated rhodopsin. Receptors were either phosphorylated by βARK or not before the assay. COS-7 cells were transfected with pBC12 (control), pBCβ-arrestin, or pBCarrestin by the DEAE-dextran method (10). The cells of one petri dish each were harvested 48 hours later and lysed in 1 ml of 5 mM tris-HCl, 2 mM EDTA (containing as protease inhibitors phenylmethyl sulfonyl fluoride at 100 μM, leupeptin at 5 μg/ml, and benzamidine at 10 μg/ml). The lysate was centrifuged at 450,000*g* for 30 min, the supernatant was washed twice with NT buffer (100 mM NaCl, 10 mM tris-HCl, pH 7.4), containing protease inhibitors, and brought up to 500 μl with NT buffer to give the cytosol preparations. Purified (>95 percent homogeneity), reconstituted β_2-adrenergic receptors from hamster lung (*5, 15*) were phosphorylated with purified βARK (>70 percent homogeneity) (*16*) to >4 mol of phosphate per mole receptor (*2, 5*). Purified (>90 percent homogeneity) G_s from human erythrocytes (*17*) was added to the vesicles, and β_2-receptor stimulation of G_s was measured as the difference of GTPase activity in the absence or presence of 10 μM (−) isoproterenol (*5*). Each reaction contained 50 fmol of β_2-receptor, 25 fmol of G_s, and 1 μl of cytosol preparation. The reactions were done at 30°C for 30 min. Rod outer segments (*18*) were disrupted in the dark with a Polytron homogenizer. Phosphorylation of rhodopsin by endogenous rhodopsin kinase, and measurement of rhodopsin-induced stimulation of transducin were done in a single reaction (*6*), containing 50 fmol of rhodopsin, 25 fmol of purified transducin (*19*), 1 μl of cytosol preparation, 500 μM ATP plus the GTPase-assay reagents (*4*) (30 min at 30°C under bright white light). Rhodopsin-induced GTPase activity was measured as the difference of activities with or without rhodopsin.

Cytosol from cells transfected with	Receptor-stimulated G protein–GTPase (mol P_i per mole of G protein per minute)		
	β_2 receptors/G_s		Rhodopsin-transducin phosphorylated
	Phosphorylated	Control	
β-arrestin	0.19 ± 0.02*	0.27 ± 0.01	0.18 ± 0.01
Arrestin	0.30 ± 0.02	0.28 ± 0.03	0.08 ± 0.03*
Control	0.29 ± 0.01	0.28 ± 0.02	0.19 ± 0.01
No cytosol	0.29 ± 0.02	0.29 ± 0.03	0.19 ± 0.03

*$P < 0.05$ versus control by analysis of variance, mean ± SEM, $n=3$.

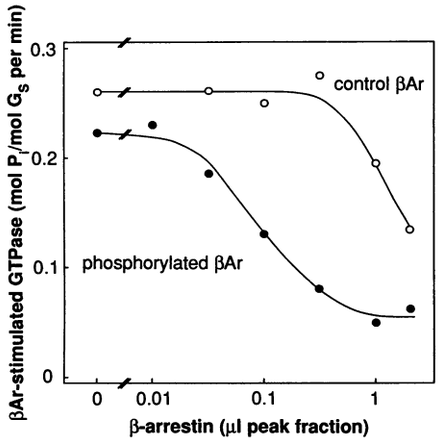

Fig. 2. Inhibition of β_2-receptor function by partially purified β-arrestin. The β_2-receptor function was assessed as the capacity to stimulate G_s (Table 2), with βARK-phosphorylated or nonphosphorylated (control) receptors. Various amounts of the peak fraction after Mono-Q chromatography and Superose-12 chromatography were added to the incubation mixture. Data are from a single experiment, with similar results obtained in two other experiments with separate β-arrestin preparations.

βARK-phosphorylated receptors by more than 75 percent and showed a 20- to 40-fold preference for βARK-phosphorylated over nonphosphorylated β receptors (Fig. 2).

Size and localization of β-arrestin mRNA were analyzed in RNA (Northern) blots of poly(A)-selected RNA from various bovine tissues. Three major mRNA species of approximately 1.3 kb, 4.1 kb, and 7.5 kb and a minor species of 2.4 kb were identified in brain and other tissues (Fig. 3). All species were detected with three different probes representing a 5′ portion of coding sequence (Fig. 3), the full coding sequence (a 1243-bp Bam HI–Apa I fragment), or a probe containing essentially only 3′ noncoding sequence (a 550-bp Kpn I–Pvu II fragment). This suggests that the different mRNA species are due to alternative processing of the same mRNA, but the exact nature of these mRNA's remains to be elucidated. Alternative RNA processing as the reason for multiple bands on the Northern blots was also suggested by DNA (Southern) analysis of genomic DNA: probing Bam HI plus Eco RI–digested bovine genomic DNA with a 354-bp Bam HI–Eco RI fragment of β-arrestin cDNA revealed a single band of ~350 bp.

β-Arrestin mRNA was found in all tissues examined, with highest levels in brain, heart, and lung, and lower levels in liver (Fig. 3). Moderate levels were found in spleen, skeletal muscle, adrenal, ovary, and testis. In the brain high levels were found in cortex and hypothalamus, and moderate levels in cerebellum, basal ganglia, brain stem, and pituitary. Overall, the distribution of the different mRNA sizes followed the same pattern, but slight variations (heart and lung) suggest possible tissue differences in RNA-processing. The widespread distribution of β-arrestin mRNA in tissues supports the suggestion that arrestin-like immunoreactivity may occur in nonretinal tissues (*11*). Since we could not find arrestin mRNA in any tissue except retina, this immunoreactivity must represent β-arrestin rather than arrestin. There are remarkable parallels between the tissue distribution of the mRNA's for β-arrestin (our data) and those for βARK (*4*). The concentrations of βARK mRNA are highest in brain, and are lower in spleen, heart, and lung; the distribution in the brain is similar to that, shown above, for β-arrestin. These parallels suggest that the two proteins may act in concert.

There is ample evidence that receptor phosphorylation is a primary mechanism leading to desensitization (*1*). Specific kinases appear to have evolved for specific G protein–coupled receptors in a variety of cells. These include β-adrenergic receptors and rhodopsin (*1*), the α-mating factor receptor in yeast (*12*), and the cyclic adenosine monophosphate (cAMP) receptor in *Dictyostelium discoideum* (*13*). All these phosphorylation processes require agonist occupancy of the respective receptors, which presumably underlies the homologous pattern of desensitization caused by these kinases. Disruption of the βARK pathway by removal of the putative phosphorylation sites on the receptors or by inhibitors of βARK essentially abolishes homologous desensitization (*3*).

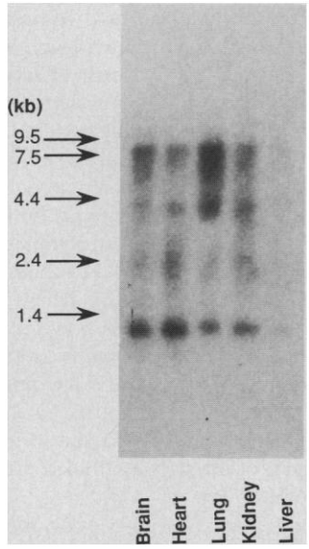

Fig. 3. Northern (RNA) blot analysis of β-arrestin mRNA in bovine tissues. Poly(A)-selected RNA (5 μg) from various bovine tissues were denatured with glyoxal and dimethyl sulfoxide, fractionated on 1% agarose gels, and transferred to a nylon membrane. The blot was hybridized with a nick-translated 354-bp Bam HI–Eco RI fragment from the 5′ portion of the coding sequence of β-arrestin cDNA in 5× SSC, 50 percent formamide, 10 percent dextran sulfate, 1× Denhardt's solution, 50 mM Na-phosphate buffer, pH 6.5, 0.5 percent SDS, and 100 μg of sheared salmon sperm DNA per milliliter. The blot was washed in 0.2× SSC, 0.1 percent SDS, at 65°C, and subjected to autoradiography for 2 days at −80°C.

Our data suggest that β-arrestin is required to effect inhibition of receptor function subsequent to phosphorylation by βARK. That this is the physiological function of β-arrestin is based on the following observations:

1) β-Arrestin is more effective than arrestin in inhibiting β-receptor function, whereas the reverse is true for rhodopsin function.

2) β-Arrestin is 20 to 40 times more effective in inhibiting the function of βARK-phosphorylated β receptors as compared to nonphosphorylated receptors.

3) The tissue distribution of β-arrestin mRNA parallels that of βARK mRNA.

We propose that homologous desensitization proceeds in the following manner: agonist occupancy of β receptors transforms them into substrates for βARK. β-Arrestin binds to the βARK-phosphorylated receptors and thereby prevents their interaction with G_s. Thus it appears that β-arrestin plays a major and very specific role in regulating the sensitivity of β-adrenergic and possibly other G protein–coupled receptors.

REFERENCES AND NOTES

1. T. K. Harden, *Pharmacol. Rev.* **35**, 5 (1983); J. L. Benovic, M. Bouvier, M. G. Caron, R. J. Lefkowitz, *Annu. Rev. Cell. Biol.* **4**, 405 (1988).
2. J. L. Benovic, R. H. Strasser, M. G. Caron, R. J. Lefkowitz, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2797 (1986).
3. M. J. Lohse, M. G. Caron, R. J. Lefkowitz, J. L. Benovic, *ibid.* **86**, 3011 (1989); W. P. Hausdorff *et al.*, *J. Biol. Chem.* **264**, 12657 (1989).
4. J. L. Benovic, A. DeBlasi, W. C. Stone, M. G. Caron, R. J. Lefkowitz, *Science* **246**, 235 (1989).
5. J. L. Benovic *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8879 (1987).
6. U. Wilden, S. W. Hall, H. Kühn, *ibid.* **83**, 1174 (1986).
7. T. Shinohara, *ibid.* **84**, 6975 (1987); K. Yamaki, Y. Takahashi, S. Sakuragi, K. Matsubara, *Biochem. Biophys. Res. Commun.* **142**, 904 (1987).
8. M. Kozak, *Microbiol. Rev.* **47**, 1 (1983).
9. S. B. Masters, R. M. Stroud, H. R. Bourne, *Protein Eng.* **1**, 47 (1986); S. R. Holbrook and S. H. Kim, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1751 (1989).
10. B. R. Cullen, *Methods Enzymol.* **71**, 684 (1987).
11. M. Mirshahi *et al.*, *FEBS Lett.* **258**, 240 (1989).
12. J. E. Reneke, K. J. Blumer, W. E. Courchesne, J. Thorner, *Cell* **55**, 221 (1988).
13. R. A. Vaughan and P. N. Devreotes, *J. Biol. Chem.* **263**, 14538 (1988); K. Meier and C. Klein, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2181 (1988).
14. D. T. Jones and R. R. Reed, *J. Biol. Chem.* **262**, 14241 (1987).
15. J. L. Benovic, R. G. L. Shorr, M. G. Caron, R. J. Lefkowitz, *Biochemistry* **23**, 4510 (1985).
16. J. L. Benovic, W. C. Stone, M. G. Caron, R. J. Lefkowitz, *J. Biol. Chem.* **264**, 6707 (1989).

17. J. Codina *et al.*, *ibid.* **259**, 5871 (1984).
18. U. Wilden and H. Kühn, *Biochemistry* **21**, 3014 (1982).
19. W. Baehr, E. A. Morita, R. J. Swanson, M. L. Applebury, *J. Biol. Chem.* **257**, 6452 (1982).
20. We thank R. Dixon for the cDNA-library; T. Shinohara, for providing pBCarrestin (*6*); B. Cullen for a sample of pBC12/CMV/IL-2 (*9*); H. Dohlman, P. Falardeau, P. Schnitzler, W. Lorenz, J. Arrizza, S. Senogles and J. Pitcher for advice; C. Stone, G. Irons, and K. Daniel for technical assistance with the transfections, cell culture, purification of βARK and β_2 receptors, and parts of the sequencing; and D. Addison for secretarial assistance. Supported in part by NIH grants HL16037 and DK19318 and a fellowship (M.J.L.) from the Deutsche Forschungsgemeinschaft.

13 March 1990; accepted 1 May 1990

An RNA Polymerase II Transcription Factor Shares Functional Properties with *Escherichia coli* σ^{70}

JOAN WELIKY CONAWAY AND RONALD C. CONAWAY

A mammalian transcription factor, which, along with other factors, is essential for accurate initiation of transcription from promoters by RNA polymerase II, has been found to regulate the interaction of polymerase and DNA. This factor, designated βγ, drastically reduces the affinity of RNA polymerase II for free DNA containing either promoter or nonpromoter sequences. In this respect, βγ functions as does the bacterial transcription initiation factor σ^{70}, which expedites the binding of *Escherichia coli* RNA polymerase to promoters in part by accelerating dissociation of the polymerase from nonpromoter sites in DNA.

INITIATION OF MRNA SYNTHESIS IS A key control point in the expression of many eukaryotic genes. Such diverse signals as heat shock, glucocorticosteroids, and lymphokines regulate the expression of genes by controlling when and how often RNA polymerase II initiates and synthesizes their mRNA. As a consequence, our ultimate understanding of eukaryotic gene regulation relies on the ability to understand, at the molecular level, how RNA polymerase II recognizes, binds to, and initiates transcription from its promoter.

Transcription initiation is an elaborate biochemical process governed by interactions between RNA polymerase II, multiple accessory transcription factors (*1–6*), an adenosine triphosphate (ATP)–deoxyadenosine triphosphate (dATP) [ATP(dATP)] cofactor (*7, 8*), and promoter DNA. Although the mechanism of initiation is poorly understood, a working model has emerged from analyses of partially purified transcription systems. This model proposes that one or more accessory factors bind directly to promoter sequences to form the first stable intermediate in initiation. RNA polymerase II, assisted by additional factors, then recognizes and assembles with this "initial" complex to form a functional preinitiation complex (*2, 5, 9, 10*). In a step requiring an ATP(dATP) cofactor, the preinitiation complex is then converted to an "activated" complex, which is capable of initiating RNA synthesis rapidly after addition of the remaining ribonucleoside triphosphates (*8*).

To define the roles played by the accessory transcription factors in initiation, we have assembled a highly purified, reconstituted RNA polymerase II transcription system from rat liver (*3–6*). Synthesis of accurately initiated transcripts in this system requires, in addition to RNA polymerase II, five accessory factors that comprise two functional classes: (i) "promoter recognition" factors (τ and ε), which interact with template DNA to form the initial complex at the promoter (*6, 11*) and (ii) "RNA chain initiation" factors (α, βγ, and δ), which subsequently, along with RNA polymerase II, assemble the functional preinitiation complex. Although no α, βγ, or δ is required for assembly of the initial complex by τ and ε, each factor ultimately becomes an integral component of the fully assembled preinitiation complex (*10*).

In *Escherichia coli*, transcription initiation factor σ^{70} regulates the binding of RNA polymerase to promoters. Biochemical studies have established that σ^{70} not only increases the affinity of polymerase for promoter sequences, but also dramatically decreases the affinity of polymerase for nonpromoter sequences (*12*). In the absence of σ^{70}, the bacterial "core" polymerase binds DNA avidly but nonspecifically, with a half-life of approximately 60 min at 25°C. In the presence of σ^{70}, RNA polymerase binds weakly to nonpromoter sequences, but binds to promoter sequences at least 10^4-fold more strongly and with a half-life of nearly 60 hours. Thus, by ensuring that *E. coli* RNA polymerase binds tightly to its promoter but weakly to nonpromoter sites in DNA, σ^{70} markedly accelerates assembly of an active preinitiation complex.

Like the bacterial core polymerase, mammalian RNA polymerase II has been found to bind stably to free DNA (*13*). In the course of our investigations, however, we observed that excess free DNA added after formation of the initial complex by τ and ε did not prevent assembly of RNA polymerase II into the functional preinitiation complex in the presence of transcription factors α, βγ, and δ (*5, 14*). This result suggested that one or more of these liver transcription factors might act to prevent formation of nonproductive binary complexes of polymerase and free DNA.

To explore this possibility further, we developed an electrophoretic mobility shift assay, based on the procedure of Fried and Crothers (*15*), to monitor formation of binary complexes of RNA polymerase II and free DNA. As shown in Fig. 1, purified RNA polymerase II (*16*) will bind strongly to a 60-bp DNA fragment containing the

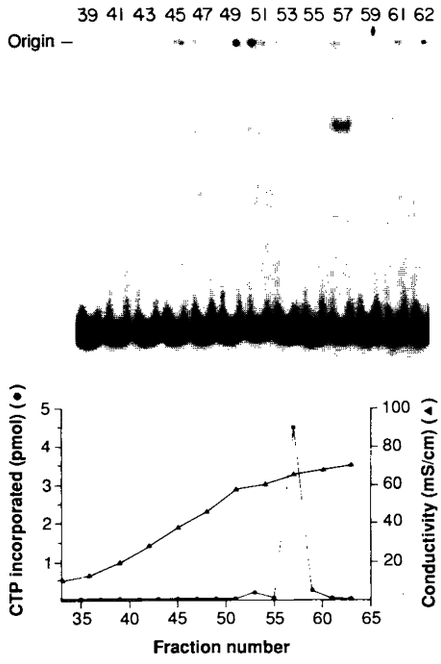

Fig. 1. Cochromatography of RNA synthesis and DNA-binding activities during TSK DEAE-NPR HPLC of RNA polymerase II. TSK DEAE-NPR HPLC was carried out as described (*16*). (**Top**) Binding reactions (*24*) contained 250 ng of Nde I–cut pUC18, 5 ng of a 60-bp [5′-^{32}P]-labeled DNA fragment containing the adenovirus −50 to +10 sequence [Ad(−50 to +10)] (*5*), and 0.2 μl of the indicated fraction. (**Bottom**) RNA synthesis reactions were performed as described (*16*) with heat denatured calf thymus DNA as the template. Conductivity was measured with a Radiometer-Copenhagen CDM-83 conductivity meter.

Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.