holic-OH, and carboxyl groups in piscidic acid and its derivatives. We compared the ability of these compounds to release P from $FePO_4$ at *p*H 4.5, a *p*H to be expected in the rhizosphere. The P-releasing ability of dimethyl fukiic acid was similar to that of piscidic acid (Table 5). This result shows that the phenolic-OH group is not related to chelation with Fe^{3+}. Trimethyl fukiic acids, where alcoholic-OH groups are replaced by methoxyl groups, have a lesser ability than piscidic acid to release P (Table 5). Thus the interrelations between the –OH and –COOH groups of the tartaric portion are the active components, perhaps acting by chelating Fe^{3+}. Further studies are required to determine the actual method of P release from an Alfisol. Also the question of how much piscidic acid and its derivatives are secreted, and at which stage of pigeon pea growth, must be investigated.

These findings imply that there are several advantages to introducing pigeon pea into low-input agriculture in the tropics. First, pigeon pea can grow and yield well in soils of low available P level and without P fertilizer applications because of its ability to tap Fe-P. Second, the available P pool in Alfisols and other related soils may be increased by the introduction of pigeon pea. Pigeon pea can utilize occluded Fe-P, which cannot be easily utilized by the other crops, as well as more soluble forms of soil P. Consequently, the successive crop may have access to such P from the residues or former rhizosphere soil of pigeon pea. Third, pigeon pea is usually cultivated as an intercrop with companion crops such as sorghum. There are indications that pigeon pea, because of its ability to utilize P from Fe-P, does not unduly compete with companion crops for fertilizer P or other sources of available P such as Ca-P. For example, we conducted a pot experiment with a similar Alfisol (1 kg per pot) of low P availability as a model system of intercropping pigeon pea and sorghum. Pigeon pea grown alone and sorghum grown alone could take up 5.18 and 4.10 mg of P per pot, respectively, from the Alfisol without P application. However, 8.32 mg of P per pot (5.27 mg from pigeon pea and 3.05 mg from sorghum) was recovered from a pot in which pigeon pea and sorghum were grown together. This observation indicates that there is little competition between sorghum and pigeon pea for P uptake from soil.

In view of the likely increasing cost and scarcity of soluble P fertilizers, especially for resource-poor farmers in marginal environments, a search for pigeon pea genotypes or other crop species with high efficiency in the use of relatively insoluble P sources would seem a worthwhile endeavor.

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RNA Polymerase II Transcription Blocked by *Escherichia coli Lac* Repressor

ULRICH DEUSCHLE,* ROBERT A. HIPSKIND, HERMANN BUJARD†

A reversible block to RNA polymerase II transcriptional elongation has been created with a *lac* operator sequence in the intron of the SV40 large T-antigen gene. When this transcription unit is injected into rabbit kidney cells expressing *Escherichia coli lac* repressor, T-antigen expression is reduced. This effect is not observed in cells lacking repressor or in the absence of the operator, and it is reversed by an inducer of the *lac* operon, namely isopropyl thiogalactoside (IPTG). In an extract of HeLa nuclei supplemented with *lac* repressor, this and similar constructs give rise to shortened transcripts that map to the 5′ boundary of the repressor-operator complex. These shorter RNAs are also sensitive to IPTG induction. This model system shows that a protein-DNA complex can block the passage of RNA polymerase II, and offers some insight into the control of eukaryotic gene expression during transcription elongation, a phenomenon observed in a variety of systems.

EUKARYOTIC GENE EXPRESSION IS A highly regulated process generally controlled at transcription initiation. Control of transcription can also be exerted during elongation of nascent transcripts (*1*). In several viral systems, RNA polymerase II pauses at specific sites both in vivo and in vitro, and distinct, prematurely terminated transcripts have been detected (*2, 3*). In HIV (human immunodeficiency virus) gene expression, this attenuation can be suppressed by the *tat* protein, leading to the generation of longer, functional transcripts (*4*). Blockage of RNA polymerase II elongation has been found in *Drosophila* (*5*) and in several vertebrate proto-oncogenes (*6–8*). The mechanisms involved are unclear, although both potential RNA secondary structure and a direct physical block have been implicated (*3, 6, 8*). We now show that the combination of *lac* repressor and its binding site, when placed far downstream of the transcriptional initiation site, can block eukaryotic gene expression in vivo and leads to premature transcription termination in vitro. This result suggests that RNA polymerase II cannot pass the complex of repressor and operator, which may offer a mechanism for gene regulation in eukaryotic cells.

To create a reversible biological impediment to transcribing RNA polymerase that could be tested both in vivo and in vitro, we used the simian virus 40 (SV40) early region, which encodes the large T-antigen (T-Ag), with transcription being driven by either the SV40 early promoter and enhancer (P_e) or the adenovirus 2 major late promoter (Ad2 MLP) [Fig. 1, construct C1 (*9*)]. In both constructs, a symmetrical *lac* operator was introduced into the large T intron [Fig. 1, construct C2 (*9*)], allowing formation of a repressor-operator complex that should be spliced out from the T-Ag mRNA. There is no evidence that *lac* repressor, bound outside a eukaryotic promoter as

Zentrum für Molekulare Biologie, Universität Heidelberg, INF 282, D-6900 Heidelberg, Federal Republic of Germany.

*Present address: Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y. 11724.
†To whom correspondence should be addressed.

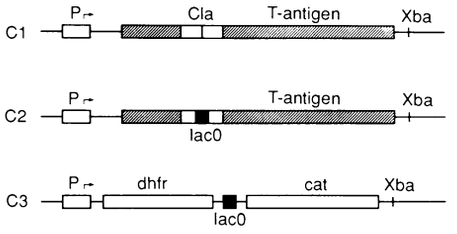

Fig. 1. DNA constructs utilized. In constructs C1 and C2 the SV40 early region is under the control of either the SV40 P_e or the Ad2 MLP (*9*). In construct C2, the *lac* operator has been inserted into an engineered Cla I site in the large T-antigen intron ~520 bp downstream of the transcription initiation site (construct C2), while construct C1 lacks the operator. Construct C3 contains a wild-type *lac* operator located between the dihydrofolate reductase coding unit (*dhfr*) and the chloramphenicol acetyltransferase gene (cat) with the Ad2 MLP driving transcription (*24*).

in these constructs, affects transcription initiation, unlike eukaryotic transcription regulatory proteins.

When microinjected into the nuclei of RKT3 cells, the SV40 early region (construct C1-SV40 P_e) leads to the synthesis of T-Ag [Fig. 2 (*10*)]. Similarly, T-Ag is detectable when the same DNA (construct C1) is tested in RKT3i cells, which constitutively express the *lac* repressor (*lac* R^+ in Fig. 2) (*10, 11*). However, a significant decrease in T-Ag is observed when the DNA containing the operator sequence in the intron (construct C2) is injected into the RKT3i cells (Fig. 2). This is not seen with construct C2 in the RKT3 (*lac* R^-) cells. Induction of the *lac* repressor by IPTG reverses the effect of construct C2 in the *lac* R^+ cells; that is, the level of T-Ag is again that found in the control cells or with construct C1. A similar effect is observed when constructs C1 and C2 are transiently transfected into these two cell lines or by coinjection of purified *lac* repressor into RKT3 cells (*12*). Thus there is a decrease in T-Ag expression in vivo that is dependent upon *lac* repressor and operator, and is reversed by the addition of IPTG. Since the operator is present in the large T intron, the effect would appear to occur during elongation of transcription. This has been tested in vitro by transcription in an extract of HeLa cell nuclei (*13*).

In vitro transcription of constructs C1 and C2 that had been linearized resulted in a long, template-dependent runoff RNA (arrow in Fig. 3A; compare lane 1 with lanes 2 to 7) (*14*). Addition of *lac* repressor had no effect on transcription from template C1 (Fig. 3A, lane 2), whereas with template C2 a new RNA of ~517 nucleotides (nt) appeared (Fig. 3A, lanes 5 and 6). The end of this shorter transcript corresponds to the 5′ boundary of the *lac* operator in the large T intron, and the transcript is no longer observed after *lac* repressor induction with IPTG (lane 7). Induction has no effect on synthesis from template C1. Both transcripts are sensitive to low concentrations of α-amanitin (lane 8), demonstrating that they are products of RNA polymerase II, unlike the RNA migrating faster than 396 nt (compare lanes 8 and 9). Since the SV40 early promoter and enhancer are relatively weak in our in vitro transcription system, the experiments were repeated with the adenovirus 2 major late promoter directing transcription of templates C1 and C2 (*9*). As above, a long runoff transcript is seen with both constructs (Fig. 3B, upper arrow). Template C1 is unaffected by either repressor or IPTG (Fig. 3B, lanes 1 and 2), while template C2, in the presence of repressor, gives rise to an abundant 530-nt RNA (Fig. 3B, lanes 4 and 5) that is again IPTG-sensitive (Fig. 3B, lane 6). To demonstrate that this premature termination is independent of the template but dependent on the position of the repressor-operator complex, an additional construct was tested [Fig. 1, C3 (*9*)] in which the wild-type *lac* operator was inserted between the dihydrofolate reductase and CAT coding regions. In the presence of the *lac* repressor, a 740-nt RNA appears (Fig. 3C, lane 2) that is not observed in the absence of repressor or in the presence of both repressor and IPTG (Fig. 3C, lanes 1 and 3). Once again the transcript

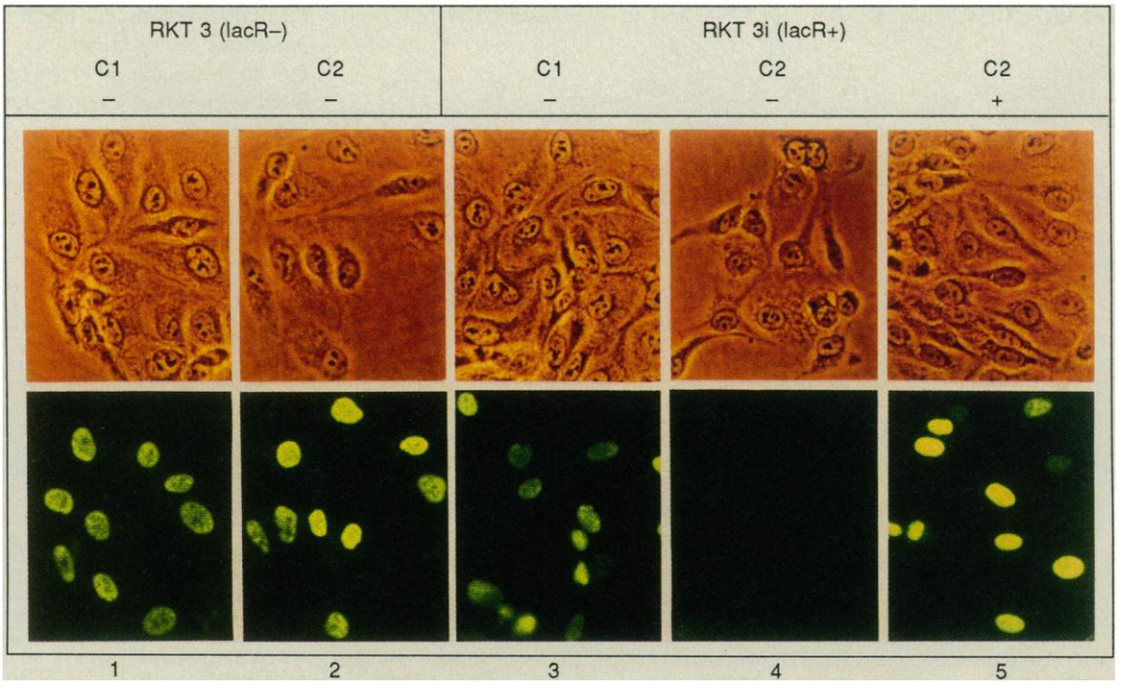

Fig. 2. *Lac* repressor blocks T-antigen expression in vivo. Plasmid constructs C1 and C2, which either lack or contain a *lac* operator in the large T-antigen intron and are under the control of the SV40 early promoter (Fig. 1), were microinjected into the nuclei of RKT3 cells that do not contain the *lac* repressor (*lac* R^-, panels 1 and 2) and RKT3i cells that constitutively express the protein (*lac* R^+, panels 3 to 5) (*11*). Induction by IPTG was used to reverse repressor binding in the last panel (+; −, no IPTG). The upper row of photomicrographs shows a field of microinjected cells under phase contrast optics and the lower row the corresponding fluorescein isothiocyanate–immunostaining against large T-antigen. Only a small proportion of the microinjected cells is shown.

is terminated near the 5′ boundary of the *lac* operator sequence.

The in vivo results show a *lac* repressor–dependent decrease in the expression of T-Ag when the operator is located ~530 bp downstream of the transcription initiation site in an intron. In this position it is unlikely that the operator sequence has an effect on either mRNA stability or translation. In addition, neither the repressor alone nor its combination with the operator apparently interferes with transcription initiation in vitro (Fig. 3). Thus, our data suggest that the repressor-operator complex blocks the passage of RNA polymerase II. The complex between the repressor and operator leads to shortened transcripts in vitro, the size of which corresponds to the distance from the promoter to the 5′ boundary of the operator but is independent of the identity of the promoter or transcription unit utilized. Furthermore, these transcripts appear at the expense of the full-length runoff RNA transcripts (Fig. 3B), thereby suggesting that a significant proportion of the RNA polymerase molecules have terminated prematurely near the position of the repressor-operator complex. From these experiments alone we cannot determine whether the shorter RNA transcripts result from actual termination events or from transcript release by an RNA polymerase II molecule blocked by the repressor-operator complex. If the latter is the case, the enzyme must release from the template before the subsequent polymerase molecule arrives, since no discrete shorter transcripts are observed that would correspond to a second blocked polymerase. In this extract, such transcripts are seen with the Ad2 MLP linked to the G-free cassette, which, along with other data, is strongly suggestive of multiple rounds of initiation in vitro (*15*). This, together with the results reported above, suggests that, regardless of the mechanism involved, the repressor-dependent blockage is functionally equivalent to actual termination.

A repressor-operator complex, when placed immediately upstream or downstream of the transcription initiation site, can interfere with eukaryotic gene expression in vivo (*16*). In the former case, the complex probably physically prevents the polymerase from initiating RNA synthesis. The latter situation may reflect the phenomenon we describe, rather than less efficient translation induced by the operator hairpin structure at the 5′ end of the mRNA, as has been suggested (*16*). Conversely, a repressor-operator complex appears to have little effect on the function of an enhancer when located between the enhancer and a promoter (*17*). As might be expected, such a complex has different effects depending upon its position.

Transcription by RNA polymerase II is thought to be highly processive, and consistent with this is its ability to displace histones in vitro (*18*). Termination can occur efficiently after the polymerase has transcribed through a functional polyadenylation signal (*1, 19*), and in one instance it has been shown that the presence of a CCAAT-box sequence further increases termination, but in an orientation-dependent manner (*20*). This could help explain the paradox of why factors bound intragenically do not necessarily lead to premature termination of transcription (*21*). However, we have shown that a *lac* repressor bound to its operator is sufficient to lead to prematurely terminated transcripts. The ability of a simple protein-DNA complex to reversibly block RNA polymerase II may serve as a paradigm for termination, as well as for transcriptional control at the level of RNA elongation. Some examples include the *Drosophila* HSP 70 gene (*5*), the human gastrin gene (*22*), attenuation in HIV (*4*), and transcriptional blocks in the protooncogenes *fos*, *myb*, and *myc* (*6–8*). In particular, the first intron of the c-*myc* gene contains a site at which RNA polymerase II can be reversibly attenuated, and both sequence and factors apparently participate in this process (*6, 23*). Our model offers one possible explanation for this, namely simple interruption of transcription elongation by a DNA-bound protein. In *E. coli* the *lac* repressor-operator complex efficiently terminates transcription by blocking elongating RNA polymerase (*24, 25*), and this is dependent upon the orientation of the wild-type *lac* operator (*26*). Moreover the complex has no effect on polymerases from phages T3 and T7 (*27*). This indicates that the interruption of transcription cannot be simply explained by a tightly bound protein forming a nonspecific block to elongation. Nevertheless, inserting a *lac* operator into introns of eukaryotic genes offers the possibility of specifically regulating their activity independently of transcription initiation.

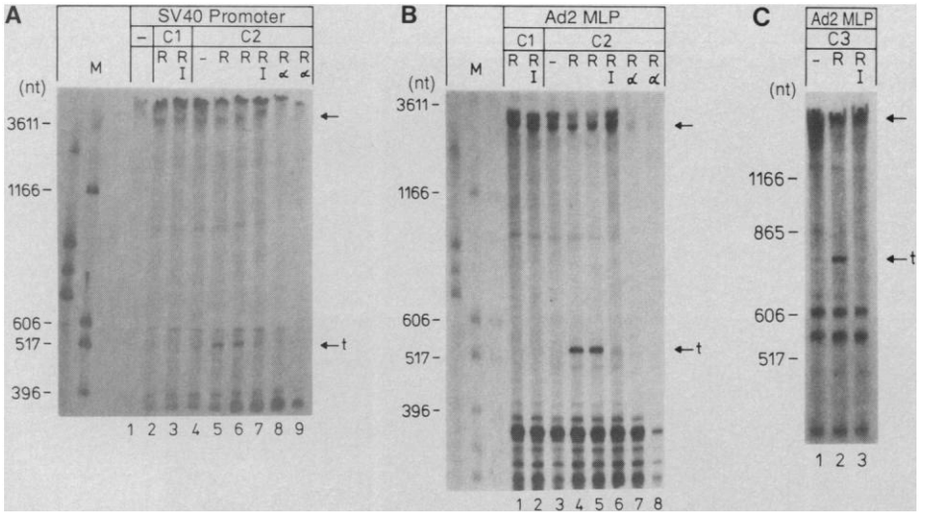

Fig. 3. Blockage of RNA polymerase II at a *lac* repressor-operator complex in vitro. Constructs C1 and C2 were linearized and transcribed in a HeLa nuclear extract (*13, 14*). (**A**) In vitro transcription from the SV40 P_e. (**B**) In vitro transcription under the control of the Ad2 MLP. The upper arrow shows the position of the runoff transcripts and the lower arrow, labeled t, the prematurely terminated RNA. A, lane 1, contains no added template. Reactions containing the repressor are labeled R and I indicates reactions where repressor binding was prevented with 500 μM IPTG. α-Amanitin was added to 0.1 μg/ml (A, lane 8 and B, lane 7) and 10 μg/ml (A, lane 9 and B, lane 8). RNA polymerase III is responsible for the RNA transcripts shorter than 396 nt in (B) (*12*). (**C**) In vitro transcription of construct C3 under the conditions described above. In all instances, the prematurely terminated RNA is dependent upon a repressor-operator complex.

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9. The region of SV40 in constructs C1 and C2 (Fig. 1) contains the Hind III to Bam HI fragment covering the early region of the virus. A Cla I site was inserted at the Taq I site at position 4759 within the large T-antigen intron, and a symmetical 20-bp *lac* operator sequence was cloned into this site in construct C2. Transcription was controlled by either the SV40 early promoter and enhancer or by the adenovirus 2 major late promoter subcloned as an Acc I to Sca I fragment (spanning positions −284 to +47) upstream of the SV40 Stu I site. The center of

the operator sequence is located ~530 bp downstream of the initiation site with the SV40 P_e and ~550 bp from the MLP transcriptional start. Construct C3 (Fig. 1) has been previously described (*24*) and the operator is centered 752 bp downstream of the RNA start site.
10. In each experiment each of 150 individual cells [rabbit kidney cell lines RKT3 and RKT3i (*11*)] was injected with 0.2 pl of DNA at 1 μg/ml (between 5 and 20 molecules per sample) with a Zeiss Automated Injection System. For induction, 50 m*M* IPTG was present in the DNA solution, and the medium contained 10 m*M* IPTG. The cells were fixed 12 hours after injection and stained with mouse antibody to T-antigen and rabbit antiserum to mouse immunoglobulin G conjugated with fluorescein isothiocyanate (FITC).
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A 49-Kilodalton Phosphoprotein in the *Drosophila* Photoreceptor Is an Arrestin Homolog

TAKUMA YAMADA, YUTAKA TAKEUCHI, NAOKA KOMORI, HISAO KOBAYASHI, YUTAKA SAKAI, YOSHIKI HOTTA, HIROYUKI MATSUMOTO*

The gene encoding the 49-kilodalton protein that undergoes light-induced phosphorylation in the *Drosophila* photoreceptor has been isolated and characterized. The encoded protein has 401 amino acid residues and a molecular mass of 44,972 daltons, and it shares approximately 42 percent amino acid sequence identity with arrestin (S-antigen), which has been proposed to quench the light-induced cascade of guanosine 3′,5′-monophosphate hydrolysis in vertebrate photoreceptors. Unlike the 49-kilodalton protein, however, arrestin, which appears to bind to phosphorylated rhodopsin, has not itself been reported to undergo phosphorylation. In vitro, Ca^{2+} was the only agent found that would stimulate the phosphorylation of the 49-kilodalton protein. The phosphorylation of this arrestin-like protein in vivo may therefore be triggered by a Ca^{2+} signal that is likely to be regulated by light-activated phosphoinositide-specific phospholipase C.

ALTHOUGH THE ROLE OF G PROTEIN–mediated guanosine 3′,5′-monophosphate (cGMP) hydrolysis in the visual transduction pathway of vertebrates has been elaborated (*1*), the corresponding pathway in invertebrate photoreceptors has not been clearly delineated. A 49-kD protein of *Drosophila melanogaster* undergoes light-induced, reversible phosphorylation in vivo (*2*). In *norpA* (no receptor potential A) mutants, which are likely to be defective in an intermediate process of visual transduction (*3*), the light-induced phosphorylation of this protein is blocked (*2*). The 49-kD protein is abundant (*2*) and has an epitope that has been observed in all of the photoreceptors of the compound eye (*4, 5*) as well as in the ocelli and larval photoreceptors (*4*). The epitope has been immunolocalized to the rhabdomeres and to the cell bodies and axons (*4*), and the 49-kD protein has been isolated from a *Drosophila* rhabdomere preparation (*5*). These results suggest that the reversible phosphorylation of the 49-kD protein may be important in visual transduction. We now report the molecular cloning of the gene encoding the 49-kD protein and show that its amino acid sequence resembles that of vertebrate arrestin. Arrestin has been proposed to interact with

T. Yamada, Y. Takeuchi, N. Komori, H. Matsumoto, Department of Biochemistry and Molecular Biology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.
H. Kobayashi, Y. Sakai, Y. Hotta, Department of Physics, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

*To whom correspondence should be addressed.

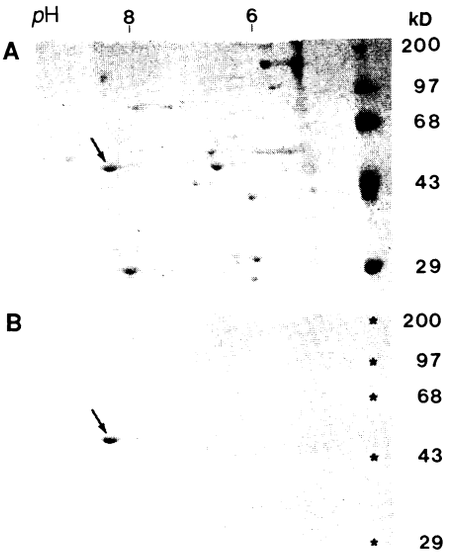

Fig. 1. In vitro translation products of poly(A)$^+$ mRNA hybrid-selected by the putative 49-kD recombinant DNA clone cTYp1-2. (**A**) Coomassie blue–stained gel. (**B**) ^{35}S autoradiogram. Approximate *p*H of the first dimension isoelectric focusing (IEF) and the molecular masses of internal markers in the second dimension [SDS–polyacrylamide gel electrophoresis (SDS-PAGE)] are indicated. The location of the 49-kD protein is indicated by arrows. A *Drosophila* cDNA library constructed in λgt11 from Canton S adult head poly(A)$^+$ mRNAs was screened with the monoclonal antibody P27 to the 49-kD protein (*4*) as a probe. One of two positive clones, cTYp1-2, was localized to the left arm of the third chromosome (66D) by in situ hybridization to the DNA of salivary gland polytene chromosomes (*28*). This cTYp1-2 clone was positively identified as representing the gene for the 49-kD protein by hybrid selection followed by in vitro translation in a rabbit reticulocyte lysate (Amersham) in the presence of [^{35}S]methionine (*29*). The translated protein sample was dissolved, together with 200 dissected eyes (dark-adapted), in an IEF lysis buffer and subjected to two-dimensional gel analysis (*2*). The identity of this spot as the 49-kD protein was confirmed by staining the immunoblot with polyclonal antibody to the 49-kD protein (*30*) and also by the fact that this spot shifts in the acidic direction upon light adaptation (*31*). The presence of a faint spot to the right of the 49-kD protein in the autoradiogram may be due to 49-kD protein phosphorylated by an endogenous protein kinase. Asterisks indicate location of markers.