to be defined, it is easiest to imagine a cis-acting role. It might, for instance, provide an entry site for ribosomes onto the mRNA, either by being a binding site or by assisting ribosomes to enter downstream from itself. However, a trans-acting process, such as ribosome modification, cannot be completely ruled out. Region P could even perform functions the ribosome itself normally accomplishes to initiate translation.

It is evident that region P of poliovirus can direct mammalian cells to translate mRNA in a cap-independent fashion. Poliovirus infection or poliovirus protein 2A expression can stop cellular cap-dependent protein synthesis, freeing ribosomes that will then translate only region P–containing RNAs. Thus, it should be possible to use region P and protein 2A to focus cellular translation on a few chosen mRNAs and increase the yield of specific proteins made in mammalian cells.

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Carboxyl Terminal Domain of $G_{s\alpha}$ Specifies Coupling of Receptors to Stimulation of Adenylyl Cyclase

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The α subunits of G_s and G_i link different sets of hormone receptors to stimulation and inhibition, respectively, of adenylyl cyclase. A chimeric α_i/α_s cDNA was constructed that encodes a polypeptide composed of the amino terminal 60% of an α_i chain and the carboxyl terminal 40% of α_s. The cDNA was introduced via a retroviral vector into S49 *cyc*$^-$ cells, which lack endogenous α_s. Although less than half of the hybrid α chain is derived from α_s, its ability to mediate β-adrenoceptor stimulation of adenylyl cyclase matched that of the normal α_s polypeptide expressed from the same retroviral vector in *cyc*$^-$ cells. This result indicates that carboxyl terminal amino acid sequences of α_s contain the structural features that are required for specificity of interactions with the effector enzyme, adenylyl cyclase, as well as with the hormone receptor.

MANY MAMMALIAN SIGNAL TRANSduction pathways use a G protein to couple hormone receptors to effector molecules. Upon interaction with an activated receptor, the α polypeptide chains of G proteins undergo a guanosine triphosphate (GTP)–dependent conformational change that allows them to activate effector enzymes or open ion channels (*1, 2*). Each member of the α chain family interacts specifically with receptors and effectors. Extensive conservation of primary structure among different G protein α chains (*1, 2*) suggests that topologically equivalent portions of each α chain may subserve the same functions in each, namely, binding and hydrolysis of guanine nucleotide, and interactions with receptors and effectors. In a recently constructed model (*3*) of a composite G protein α chain (α_{avg}), we tentatively assigned these functions to different regions of α_{avg}. In our model (*3*), regions of linear sequence that presumably contribute to the polypeptide's guanine binding site divide the rest of the chain into three potential domains. Biochemical evidence (*1, 3*) strongly suggests that the extreme COOH-terminus (domain III) directly contacts receptors. This conclusion is supported by the identification (*4*) of a mutation, located in the same region of α_s, that specifically uncouples G_s from receptors. We proposed (*3*) that domain II interacts with effectors, on the basis of studies (*5, 6*) that suggested similar roles for the corresponding regions of two other GTP-binding proteins, bacterial elongation factor Tu (EF-Tu) and the mammalian 21-kD polypeptides (p21) encoded by the *ras* oncogenes.

The high degree of conservation among nucleotide sequences of different α chains provided a simple approach to testing these functional assignments. A conserved Bam HI restriction endonuclease site, present in almost all mammalian α chain cDNAs, neatly separates domains I and II from domain III. This site allows convenient construction of chimeric cDNAs in which a hybrid polypeptide derived from two different α chains is encoded in a single reading frame. The first chimeric α chain we constructed (Fig. 1) encodes the NH_2-terminal 212 amino acids of a murine α_i chain (7), designated α_{i2} (*8*), linked to the COOH-terminal 160 residues of murine α_s (7). Whether α_{i2} is the α chain of a G protein that mediates hormonal inhibition of adenylyl cyclase is unknown. If so, the postulated locations of receptor- and effector-recognition sites predict that the chimera would couple β-adrenoceptors (interacting with domain III of the α_s portion of the chimera) to inhibition of adenylyl cyclase (mediated by the postulated effector interaction region in domain II, contributed by α_{i2}).

To investigate the function of the α_i/α_s chimera, we expressed it in S49 *cyc*$^-$ by means of a retroviral vector as previously described (*4*). We compared regulation of

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adenylyl cyclase in two independently transformed cells that express the chimera (*cis1.2* and *cis1.5*) to regulation in wild-type S49 clones (*wt*), and in two *cyc*$^-$ clones transformed (*7*) with a complete α_s cDNA (*8*) (*csw1.3* and *csw1.2*). Immunoblots with an antiserum directed against a sequence present in the COOH-terminal portion of α_s established that the transformed cells express α chains of the appropriate size (Fig. 1). The S49 *wt* cells express at least two α_s polypeptide chains, of 52 and 45 kD, which result from alternative splicing of α_s transcripts (*9, 10*). However, *csw1.3* and *csw1.2* cells express only the recombinant 52-kD form of α_s (*4*). Membranes of *cis1.2* and *cis1.5* cells contain a 46-kD polypeptide that is detected both by the α_s antiserum (Fig. 1) and by an α_i-specific antiserum (*11*) directed against a sequence from the NH_2-terminal half of α_{i2} (*12*).

Regulation of adenylyl cyclase in membranes containing the α_i/α_s chimera closely resembles regulation of the enzyme in membranes of *wt* S49 cells or cells that express wild-type α_s (Figs. 2 and 3 and Table 1). The concentrations of isoproterenol required for half-maximal stimulation of adenylyl cyclase [median effective concentration (EC_{50})] are similar in membranes of *wt*, *cis1.5*, and *csw1.3* cells (Fig. 2). Prostaglandin-E_1, which acts through a different receptor, also stimulates adenylyl cyclase in all three types of membranes (*12*). Two agents that act directly on α chains, GTPγS and NaF, stimulate adenylyl cyclase via α_i/α_s (Fig. 3). A hydrolysis-resistant GTP analog, GTPγS, causes persistent α chain activation (*1, 2*), whereas fluoride ion [complexed as AlF_4^- (*13*)] interacts with the guanosine diphosphate (GDP) form of α chains, and is thought to activate G proteins by substituting for the γ-phosphoryl of GTP (*2, 14*).

The patterns of adenylyl cyclase response to different stimulators in *cis1.2* and *cis1.5* membranes, which contain the chimeric α chain, generally resemble patterns seen in membranes from S49 *wt* cells, as well as in *csw1.3* and *csw1.2*, which contain recombinant normal (52 kD) α_s (Fig. 3). The data suggest, however, that regulation of adenylyl cyclase by the chimera differs subtly from regulation by normal α_s. Differences are seen most clearly if responses to different stimulators are compared to adenylyl cyclase activity measured in the presence of forskolin, a drug thought to stimulate adenylyl cyclase directly (*15, 16*). Relative to forskolin, responses to isoproterenol and NaF are slightly lower in membranes containing the α_i/α_s chimera than in membranes containing normal α_s (Fig. 3). The reduction in the maximal response to GTPγS is much more prominent (Fig. 3), although this GTP analog stimulates adenylyl cyclase at similar concentrations in all three membrane types (EC_{50} = 0.6, 0.2, and 0.1 μM in *cis1.5*, *wt*, and *csw1.3* membranes, respectively). The reason for relatively reduced responsiveness of the α_i/α_s chimera to GTP analogs is unknown. One possibility is that the chimeric protein binds GDP more tightly than does wild-type α_s, a property that might reduce its rate of activation.

Cholera toxin stimulates adenylyl cyclase by catalyzing adenosine diphosphate (ADP)–ribosylation of α_s, a modification that stabilizes the protein in its active GTP-bound conformation and inhibits its GTPase activity (*1, 2*). In *cis1.5* membranes, cholera toxin fails either to stimulate adenylyl cyclase activity or to catalyze detectable transfer of radiolabel from ^{32}P-labeled nicotinamide adenine dinucleotide (NAD^+) into the α_i/α_s chain (*12*). This lack of effect of cholera toxin is not surprising because the arginine residue (position 203 in α_{avg}) that is a candidate for toxin modification in the α_i/α_s chimeric protein derives from α_i rather than from α_s. Although α_i can be ADP-ribosylated by cholera toxin, this can be done only under unusual conditions (*17, 18*) and to a relatively low extent. Thus, it is likely that a structural feature derived from

Table 1. Inhibition of adenylyl cyclase activity by somatostatin. Results (measured in picomoles per milligram per minute) are reported as the mean ± SEM for triplicate determinations. Concentrations were as follows: GTP, 50 μM; ISO, 100 μM isoproterenol; forskolin, 10 μM; SST, 10 nM somatostatin. Adenylyl cyclase activity was measured as described in the legend of Fig. 3.

Treatment	Adenylate cyclase activity in:					
	*wt**	Inhibition (%)	*csw1.3*†	Inhibition (%)	*cis1.5*‡	Inhibition (%)
GTP	14.5 ± 0.5	4	10.0 ± 0.9	28	17.7 ± 1.9	38
GTP + SST	13.9 ± 0.1		7.2 ± 1.0		11.0 ± 1.6	
ISO + GTP	174 ± 10	13	225 ± 18	34	118 ± 12	48
ISO + GTP + SST	154 ± 12		149 ± 14		57 ± 6	
Forskolin	377 ± 37	13	519 ± 21	34	347 ± 15	39
Forskolin + SST	326 ± 22		344 ± 21		211 ± 9	

*The 52- and 45-kD α_S. †The 52-kD α_S. ‡The α_i/α_s.

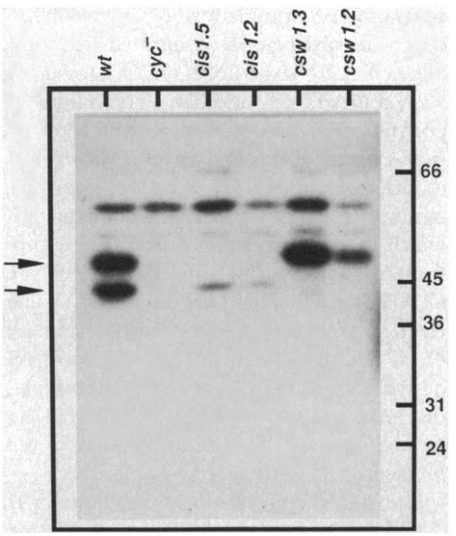

Fig. 1. Expression of recombinant α chains in S49 cells. Immunoblot analysis of *wt*, *cyc*$^-$, and transfected S49 cell membranes was performed with an antiserum to a peptide from the COOH-terminus of α_s. Arrows indicate the positions of the 52- and 45-kD forms of wild-type α_s (*23*). The positions of molecular size markers (in kilodaltons) are shown on the right. The α_i/α_s cDNA was constructed by ligating a 5′ 732-bp Eco RI–Bam HI fragment of a mouse α_{i2} cDNA clone (*7*) with a 3′ 666-bp Bam HI–Hind III fragment from a mouse α_s cDNA (*7*). The Hind III fragment was ligated into the unique Hind III restriction endonuclease site in the retroviral vector pMV7 (*24*). After passage of the vector containing the α_i/α_s cDNA through packaging cell lines, *cyc*$^-$ cells were infected, and independent transformants were isolated by cloning populations of virally infected cells in soft agar containing G418, as described (*4*) for the wild-type α_s cDNA. Membrane proteins (60 μg per lane) were resolved on an 11% SDS-polyacrylamide gel and transferred to nitrocellulose (*25*). Blots were blocked for 1 hour with a solution (Blotto) of 50 mM tris buffer, pH 7.5, 150 mM NaCl, 5% nonfat dry milk, and 0.05% Tween-20, then incubated for 2 hours in Blotto plus affinity-purified antiserum (2 mg/ml). Three 10-min washes with Blotto were followed by incubation for 1 hour with 10 to 15 mCi of ^{125}I-labeled protein A (Amersham). Blots were washed twice for 10 min with Blotto, and twice for 10 min with a TBS solution (50 mM tris buffer, pH 7.5, 150 mM NaCl, and 0.02% Tween-20). Membranes were prepared by nitrogen cavitation of cells and assayed for protein (*4*). A rabbit antiserum directed against residues 323 to 339 of α_s was produced (*26*). For affinity purification, the antiserum was incubated for 2 hours at room temperature in a suspension of α_s peptide coupled to Sepharose 4B (Bio-Rad). Unbound antibodies were removed by washing the resin with 10 ml of TBS and 10 ml of TBS plus 0.35M NaCl. Specific α_s antibodies were eluted with 2M glycine, pH 2.5, rapidly neutralized, and dialyzed overnight against TBS.

the NH_2-terminal 60% of α_{i2} limits susceptibility of the α_i/α_s chimera to modification by the toxin.

The relative efficiency of receptor-effector coupling by wild-type α_s and coupling by the α_i/α_s chimera might be estimated by comparing ratios of G_s-dependent adenylyl cyclase activity to relative amounts of α chain detected by antibody. Although immunoblot analysis showed that the levels of expression differed in different cell lines (Fig. 1), attempts to quantitate the amounts of α_s and α_i/α_s antigen in *csw* and *cis* cells showed that it was impossible to estimate relative amounts of α chain protein with precision greater than ±100 to 200% (*12*). Bearing this limitation in mind, our best estimate is that coupling efficiencies of the α_i/α_s chimera and recombinant 52-kD α_s are roughly similar; they probably do not differ by more than two- to threefold.

In wild-type and *cyc*$^-$ S49 cells, somatostatin inhibits adenylyl cyclase through activation of G_i (*19*). The data indicate that somatostatin receptors neither stimulate nor inhibit adenylyl cyclase via the α_i/α_s chimera (Table 1). As in *wt* and *csw1.3* membranes, somatostatin fails to stimulate adenylyl cyclase in *cis1.5* membranes. Conversely, the hormone inhibits adenylyl cyclase to similar extents in *csw1.3* and *cis1.5* membranes (and to a lesser extent in *wt*). Inhibition by somatostatin is mediated by endogenous G_i, because it is susceptible to blockade by pertussis toxin in all three membrane types (*12*). The chimera lacks the cysteine residue near the COOH-terminus of α_i that is ADP-ribosylated by pertussis toxin (*20*); consequently, even if the α_i/α_s chimera were capable of being activated by the somatostatin receptor, its effects on adenylyl cyclase would be resistant to pertussis toxin.

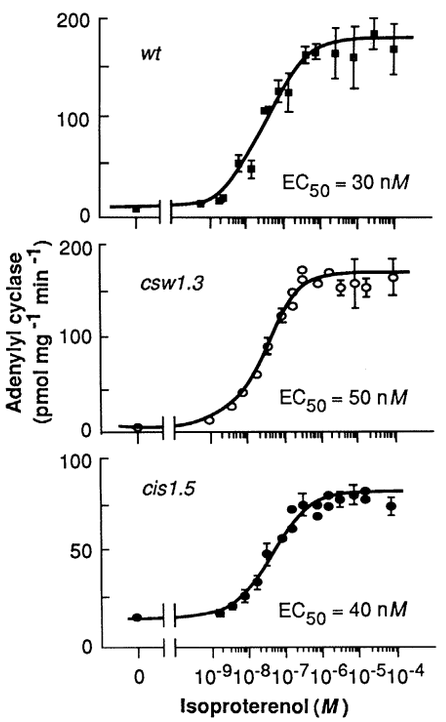

Fig. 2. Isoproterenol stimulation of adenylyl cyclase in membranes from *wt*, *csw1.3*, and *cis1.5* cells. Membranes were assayed for adenylyl cyclase activity (*4*), in a volume of 100 μl containing 50 m*M* tris buffer, *p*H 8, 2.5 m*M* $MgCl_2$, 1 m*M* EDTA, 2 m*M* β-mercaptoethanol, bovine serum albumin (1 mg/ml), 10 m*M* creatine phosphate, creatine phosphokinase (10 U/ml), 1 m*M* cAMP, 0.4 m*M* [α-^{32}P]ATP (Amersham), 50 μ*M* GTP, and the indicated concentrations of isoproterenol. Values represent means ± SD for triplicate measurements.

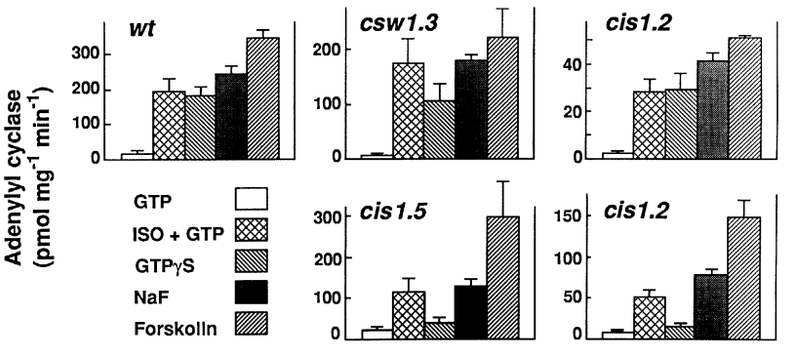

Fig. 3. Adenylyl cyclase activity in S49 cell membranes. Adenylyl cyclase activity was measured as described in Fig. 2. Values represent means ± SD for triplicate or quadruplicate determinations. In *cyc*$^-$, forskolin-stimulated activity was 72.7 ± 7.1 (n = 4) pmol mg^{-1} min^{-1}, and activities with the other activators were <2 pmol mg^{-1} min^{-1}. Activator concentrations were as follows: GTP, 50 μ*M*; isoproterenol (ISO), 100 μ*M*; GTPγS, 100 μ*M*; NaF, 10 m*M*; and forskolin, 10 μ*M*.

The present results show that the α_i/α_s chimera, like normal α_s, can mediate β-adrenoceptor stimulation of adenylyl cyclase. The observations confirmed one tenet of the α chain structure-function model (*3*) and contradicted another. The chimeric polypeptide's susceptibility to activation by β-adrenoceptors is compatible with the model's assignment (*3*) of the receptor recognition region of α chains to domain III, which the chimera derives from α_s. A presumptive amphipathic α helix at the extreme COOH-terminus has been identified as one element of the receptor recognition unit (*3, 4*). If additional structural features are required for specific recognition of receptors, our results suggest that these features are located in the COOH-terminal 40% of the α chains.

Our model suggested (*3*) that domain II contains structural determinants of effector recognition, based on the considerable variation in amino acid sequence among different α chains in this region and on analogies with EF-Tu and the *ras* proteins (*5, 6*). The phenotype of *cyc*$^-$ cells expressing the α_i/α_s chimera supports a different inference—that a region of distinctive primary structure in domain III, rather than domain II, confers on α_s the ability to stimulate adenylyl cyclase. It is not yet possible to pinpoint specific regions responsible for effector stimulation within the ~100 amino acid residues of domain III.

It is tempting to expand this narrow inference into the broader conclusion that domain II does not participate in specific recognition of effectors by G protein α chains. However, in the absence of information regarding the specific effector (or effectors) regulated by α_{i2}, we cannot rule out the possibility that peptide sequences contributed to the α_i/α_s chimera by α_{i2} do participate directly in binding of the chimera to the adenylyl cyclase, although structural features contributed by domain III specify stimulation of the enzyme. Phenotypes produced by expression of other chimeric α chains will constitute stringent tests of this qualification.

The question of the possible functions of domains I and II is particularly pressing because, for each class of α_i chain, the primary structures of these domains are highly conserved among species (>95% identical amino acid sequences) (*1, 2*). This suggests that distinctive structural features of these domains specify evolutionarily conserved interactions with other proteins. One possibility is that portions of domains I and II participate in GTP-regulated association with the βγ complexes of G proteins. In accord with this possibility, proteolytic cleavage of the α chain of retinal transducin, at a site in domain I, appears to prevent association of the remaining α chain with βγ (*21*). The ability of cholera toxin–catalyzed ADP-ribosylation of an arginine residue in domain II to reduce the affinity of α_s for binding βγ complexes (*22*) suggests that this domain also may interact with βγ. Also, G protein α chains may interact selectively with classes of proteins (such as, cytoskeletal elements) in addition to receptors, effectors, and βγ complexes.

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Viroid-Induced Phosphorylation of a Host Protein Related to a dsRNA-Dependent Protein Kinase

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Viroids are very small, unencapsidated RNAs that replicate and induce severe disease in plants without encoding for any proteins. The mechanisms by which the viroid RNA regulates these events and interacts with host factors are unknown. An M_r 68,000 host-encoded protein has been identified that is differentially phosphorylated in extracts from viroid-infected and mock-inoculated tissues. This phosphoprotein is immunologically related to a double-stranded (ds) RNA–dependent protein kinase from virus-infected, interferon-treated human cells. Further, nucleotide photoaffinity labeling indicates that the protein has an ATP binding site. This protein is similar to dsRNA-dependent protein kinases implicated in mammalian systems in the regulation of protein synthesis and virus replication.

VIROIDS COMPRISE A DISTINCT AND unique class of pathogenic agents consisting of single-stranded RNA molecules of between 250 and 400 nucleotides with a high degree of base pairing (*1*). They replicate autonomously in plants, lack any detectable mRNA activity, and have no protective capsid (*1, 2*). Significant progress has been made in determining the structure and nucleotide sequence of many viroids. Relatively little is known, however, regarding possible mechanisms of pathogenicity (*2*). Viroids replicate and induce severe disease in plants without encoding for any proteins. This suggests that the viroid RNA may interact with selected host factors to regulate replicative processes and initiate events culminating in symptom expression. Identification of viroid-associated host factors and the characterization of their role in normal and infected plants is an area of great interest but one in which little progress has been made.

Experiments presented here provide evidence for a viroid-induced alteration of a host-encoded protein. We have found that potato spindle tuber viroid (PSTV) infection of tomato plants selectively alters the phosphorylation state of an M_r 68,000 host-encoded protein (designated p68). Furthermore, several lines of evidence support the notion that this phosphoprotein has dsRNA-dependent protein kinase activity.

To determine if viroid infection alters the phosphorylation state of host cell proteins, we prepared mock-inoculated and PSTV-infected tomato tissues and dialyzed as described (*3*). Homogenates from PSTV-infected and mock-inoculated tissues were treated identically, and exactly equal aliquots of each were loaded onto polyacrylamide gels. Densitometer tracings of the Coomassie blue–stained gels indicated that equal amounts of protein were present in homogenates from PSTV-infected and mock-inoculated tissues. The ^{32}P incorporation from [γ-^{32}P]ATP into two bands in the region of M_r 68,000 was significantly elevated in extracts from PSTV-infected plants as compared to mock-inoculated plants (Fig. 1, lanes 1 and 2). This autoradiogram was exposed for only 2 hours, and the only labeled bands present were in the region of M_r 68,000. Longer exposure revealed other labeled peptides; however, under these conditions the doublet could not be resolved well. The doublet bands seen appear to be the result of differential phosphorylation of a single p68 protein, since label from the lower band could be chased into the higher band by addition of cold ATP. Petryshyn *et al.* (*4*) observed a similar phosphorylation pattern with the dsRNA-dependent protein kinase from reticulocyte lysates. Densitometer scans of the autoradiogram in Fig. 1 indicate that in homogenates from infected plants approximately threefold as much ^{32}P is incorporated into the p68 bands as into homogenates from mock-inoculated plants. Phosphorylation of p68 in tomato tissue homogenates was dependent on Mg^{2+}, stimulated by Mn^+, and independent of cAMP and cGMP. In addition, [γ-^{32}P]8-azido adenosine triphosphate (8-N_3ATP) mimicked [γ-^{32}P]ATP in phosphotransferase activity.

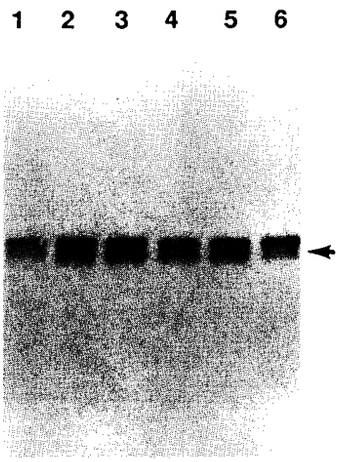

Fig. 1. Incorporation of ^{32}P from [γ-^{32}P]ATP into p68 from homogenates of PSTV-infected (lane 2) and mock-inoculated (lanes 1, 3, 4, 5, and 6) tissues. Homogenates (*3*) were incubated with 1 μM [γ-^{32}P]ATP (100 Ci/mmol), 20 mM tris-HCl (pH 7.4), 5 mM $MgCl_2$, 5 mM $MnSO_4$, and dsRNA at concentrations indicated for 10 min at 30°C. Protein solubilizing mix (*3*) was added and the mixture was heated in a boiling water bath for 4 min. Polypeptides were separated on a 10% polyacrylamide gel containing SDS, which was then fixed, stained with Coomassie blue, and autoradiographed. Arrowhead shows p68.

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