The G11 Gene Located in the Major Histocompatibility Complex Encodes a Novel Nuclear Serine/Threonine Protein Kinase*

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Protein kinases are involved in signal transduction pathways and play fundamental roles in the regulation of cell functions. Here we report that the gene G11 located in the human major histocompatibility complex encodes a novel Ser/Thr protein kinase. The G11 gene products of 41.5 and 30 kDa were expressed in insect cells using the baculovirus system and transiently in the mammalian cell line COS-7. It was found that after immunoprecipitation of the G11 polypeptides from recombinant baculovirus-infected insect cell lysates or transfected COS-7 cell lysates the immunoprecipitates contained a Mn2+--dependent protein kinase activity that phosphorylated α-casein at Ser/Thr residues and histone at Ser residues. Furthermore, mutation of the ATP-binding site by converting the invariant lysine in histone at Ser residues. Furthermore, mutation of the ATP-binding site by converting the invariant lysine in histone at Ser residues. Furthermore, mutation of the ATP-binding site by converting the invariant lysine in histone at Ser residues.

The major histocompatibility complex (MHC)† which is located on the short arm of human chromosome 6, in the 6p21.3 band, spans ~4 megabase pairs of DNA and contains the class I and class II genes that encode cell surface molecules essential for the immune response to infectious agents (1). In recent years a detailed characterization of the MHC has revealed the presence of at least 150 additional genes to the class I and class II genes that mostly encode proteins of unknown function (2–4). Characterization of these genes and the proteins they encode is of major biomedical interest as susceptibility to a large number of human diseases, including over 40 autoimmune diseases and the immunodeficiencies IgA deficiency and common variable immunodeficiency, is associated with genes within the MHC (5, 6). One of the recently described novel genes, called G11, is located in the class III region of the MHC 611 bp upstream of the complement C4A locus (7). cDNAs corresponding to two different G11 mRNAs were obtained and sequenced, the longest cDNA predicting an intracellular protein of 364-amino acids (labeled here G11-Z) (GenBank accession number L26260) (8). The second G11 cDNA predicts an intracellular protein (labeled here G11-Y) (GenBank accession number X77380) (7) which lacks the N-terminal 110 amino acids of the 364-amino acid G11 polypeptide. In addition, sequence analysis of PCR products generated by amplification of reverse transcribed RNA from a number of different cell lines has shown the presence of sequences that contained a 12-bp insertion between nucleotides 790 and 791 of the G11-Z cDNA sequence (7). This is due to the differential use of two splice sites at the end of exon 5 and results in the presence of G11-Z polypeptides of 364 or 368 amino acids, and G11-Y polypeptides of 254 or 258 amino acids, which either contain or lack the 4 amino acids, Val-Cys-Asp-Cys (7). The G11 polypeptides share limited sequence similarity with a number of different protein families, although the most significant similarity found was with the tyrosine kinase transforming protein from fujinami virus (18.7% identity, 32.5% similarity over the C-terminal 157 amino acids; align score, 3.84 S.D. units) (7). If the most extensive homology is indicative of the function of G11 then it could suggest that the G11 gene encodes a novel kinase-like activity.

The importance of phosphorylation events in both prokaryotic and eukaryotic species and in almost every aspect of nuclear as well as cytoplasmic processes has become clear in recent years. Cellular protein kinases and phosphatases participate in, among other events, the regulation of differentiation, cell division, transcription, and DNA repair through universal signaling pathways (9–12). The end result is usually the activation or inactivation of proteins that regulate transcription, resulting in specific changes in gene expression. In most cases, the mechanisms influencing gene transcription also appear to involve protein phosphorylation. The regulation can be positive or negative and it can affect DNA binding activity, transactivation function, and modulate nuclear translocation (12). Thus the characterization of all intracellular kinases and...
phosphatases is crucial in order to fully understand their function and the signaling pathways they are components of.

In this paper we report the expression of the G11 coding sequence in insect cells and in the mammalian cell line COS-7. We show that immunoprecipitates of G11 prepared from insect cells infected with the G11 recombinant virus and from COS-7 cells transfected with the G11 coding sequence possess a Ser/Thr protein kinase activity that phosphorylates the nonspecific kinase substrates casein and histone. This activity was absent when the invariant lysine (amino acid 317) in the catalytic domain was mutated to a proline. Furthermore, we present evidence that the subcellular localization of the G11 polypeptide is in the nucleus.

**EXPERIMENTAL PROCEDURES**

**PCR and Molecular Cloning**—In order to generate the complete G11-Z eDNA sequence the 429 bp corresponding to the 5' end was obtained by performing PCR on reverse-transcribed total RNA isolated from Molt4 cells by the RNazol B method (Biotecx Laboratories) using primers 5'-CGTCAAGCTTTTGTGAGGGCC-3' (sense) and 5'-GGATTCCTCTCTGACACCACAATG-3' (antisense). 10% of the product from the first round PCR was used as a template for a second round PCR. The primers were 5'-GGATCCGCGGCAAACTGTTG-3' (sense) and 5'-AAGAAGATCCGGCTCACAAGC-3' (antisense). The PCR products were end-filled and digested with EcoRI/BamHI before the 429-bp product was recovered from an agarose gel using GeneClean (Bio 101 Inc.). The pBluescript vector containing the G11-Y construct (7) was digested with EcoRI/BamHI to remove the 130 bp at the 5' end of the G11-Y cDNA and this was replaced with the 429-bp EcoRI/BamHI PCR fragment to create the full-length G11-Z cDNA. Clones were fully sequenced on both strands.

The 1220-bp full-length G11-Z cDNA. Clones were fully sequenced on both strands. EcoRI replaced with the 429-bp 9 CAATAAGTGG-3' with product from the first round PCR was used as a template for a second round PCR. The three mutant cDNAs, designated G11–300R, G11–315R, and G11–317R, were plaque-purified three times on monolayers of Sf21 cells (13). For production of G11-Z recombinant virus, Sf21 cells infected with the G11 recombinant virus at a multiplicity of infection of 20 plaque forming units/cell. Recombinant viruses were screened by a differential dot-blot assay as described in Ref. 27. Clone 1F3 (lgG1) which recognizes both conformations of the wild type and mutant forms of G11-Z equally well, as seen in SDS-PAGE gels loaded with the same amount of each immunoprecipitate, from the same number of cells transfected with the appropriate plasmid construct (data not shown) was used in kinase assays when indicated.

**Immunoprecipitation and Western Blot Analysis**—Infected and uninfected cells were lysed in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% (v/v) sodium deoxycholate, 1% (v/v) Nonidet P-40, 0.1% (w/v) SDS, 2 mM mithionine, 1 mg/ml bovine serum albumin, 0.02% (w/v) Na3P, then the expressed tagged proteins were immunoprecipitated from the lysates using the monoclonal antibody T7.Tag (Novagen), and the immune complexes were recovered on Protein A-Sepharose (14). Whole cell extracts or immune complexes were dissociated in sample buffer (0.4 M Tris-HCl (pH 6.3), 2.3% (w/v) SDS, 10% (v/v) glycerol, and 5% (v/v) 2-mercaptoethanol) and analyzed by SDS-PAGE in 12% (w/v) acrylamide gels (15). For immunoblot analysis, proteins were separated by SDS-PAGE, electrophotographed to a nitrocellulose membrane in 39 mM glycine, 48 mM Tris, 0.375% (w/v) SDS, then probed with a 1:2000 dilution of the T7.Tag monoclonal antibody as described in Ref. 16.

**Production of Anti-G11 Monoclonal Antibodies**—To produce anti-G11 monoclonal antibodies the G11-Z cDNA was subcloned into SmaI digested pGEX vector. The glutathione S-transferase-G11 fusion protein was isolated from a SDS-PAGE and used to immunize BALB/c mice. Monoclonal antibodies were screened by a differential dot-blot assay as described in Ref. 27. Clone 1F3 (lgG1) which recognizes both conformations of the wild type and mutant forms of G11-Z equally well, as seen in SDS-PAGE gels loaded with the same amount of each immunoprecipitate, from the same number of cells transfected with the appropriate plasmid construct (data not shown) was used in kinase assays when indicated.

** Protein Kinase Assays**—For protein kinase assays SF21 cells infected with the appropriate recombinant or wild-type virus, or COS-7 cells transfected with the appropriate construct, were lysed in 0.5% (w/v) SDS, 5% (w/v) EDTA, 1% (v/v) Triton X-100, then diluted in modified RIPA buffer (150 mM NaCl, 16 mM Na2PO4, 4 mM NaH2PO4, pH 7.3 (17). The expressed proteins were immunoprecipitated from the lysates using the T7.Tag monoclonal antibody or the anti-G11 monoclonal antibody and the immune complexes were recovered on Protein A-Sepharose. The Sepharose pellet was washed 5 times with the modified RIPA buffer (the SDS was omitted) and twice with 0.05 M Tris-HCl (pH 7.4). The pellet was then assayed for protein kinase activity in 10 µl of kinase buffer containing 20 mM MnCl2, 50 mM HEPES (pH 8), 1 mg/ml substrate (r-casein or histone, Sigma), and 1 µCi of [γ-32P]ATP for 10 min at 30 °C. The reaction was stopped by the addition of 30 µl of electrophoresis sample buffer unless the reactions were further manipulated as indicated. Quantitative detection of the radioactivity was performed using a PhosphoImager and ImageQuant software (Molecular Dynamics).

**Identification of Phosphoamino Acid**—Analysis of 32P-labeled protein was carried out as follows: T7-Tag labeled r-casein or histone were eluted from polyacrylamide gels by incubating crushed gel slices in 50 mM NH4HCO3, 0.1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol for 2 h at 37 °C. Eluted proteins were concentrated by trichloroacetic acid precipitation with 20% (v/v) trichloroacetic acid. The precipitate was dissolved in 6 N HCl and incubated at 110 °C for 1 h. Samples dissolved in a marker mixture containing phosphoserine, phosphothreonine, and phosphotyrosine at a concentration of 1 mg/ml each were then electrophoresed at pH 1.9 for 60 min in a buffer containing 7.8% (v/v) acetic acid and 88% (v/v) formic acid, followed by electrophoresis at pH 3.5 for 60 min in a buffer containing 5% (v/v) glacial acetic acid and 0.5% (v/v) pyridine. After electrophoresis the plates were dried, the internal phosphoamino acid markers were detected by staining with ninhydrin, and the labeled phosphoamino acids detected by autoradiography.

**Labeling of G11 with FSBA**—Insect cells infected with the G11-Y recombinant virus were lysed in modified RIPA buffer. The extract was clarified by centrifugation at 10,000 × g for 5 min. The lysates were incubated with 20 µl MclCl2, 50 µl HEPES (pH 8), 1 µl FSBA (added from a 50 µM stock made up in dimethyl sulfoxide), in the presence or absence of 1 µM ATP at 30 °C. After the reactions had been terminated by adding electrophoresis sample buffer, immunoprecipitation was carried out by 10-fold dilution of the samples in PBS containing 1% (v/v) Triton X-100, followed by incubation overnight at 4 °C with the T7.Tag monoclonal antibody. Immune complexes were recovered on Protein A-Sepharose, washed three times in PBS containing 0.2% (v/v) Tween 20 and eluted by boiling in electrophoresis sample buffer. Proteins were separated by SDS-PAGE and subjected to Western blotting. Blots were incubated with anti-FSBA antibodies at a 1:4000 dilution. Visualization of the bound anti-FSBA antibodies was achieved using the ECL detection method (Amersham), followed by autoradiography.

**Immunocytochemistry**—Immunocytochemistry analysis was carried out 48 h after transfection on COS-7 cells that had been seeded onto the plastic well of a 1-Chamber Lab-Tec slide (Nunc, Life Technologies,
Expression and Immunological Detection of the G11 Polypeptides in Insect Cells—For functional characterization of the 368-amino acid G11-Z and the 258-amino acid G11-Y polypeptides, the entire protein-coding regions including a T7 gene 10 peptide tag at the N-terminal end were expressed in Sf21 insect cells under the control of the polyhedrin promoter. As shown by SDS-PAGE analysis of Trans35S-labeled cells, G11-Z and G11-Y were expressed as 41.5- and 30-kDa intracellular polypeptides (Fig. 1A, lanes 1 and 3), respectively. These are very close to the expected molecular masses of the G11-Z (40.5 kDa) and the G11-Y (28.4 kDa) polypeptides plus the 1.5-kDa peptide tag. Time course experiments revealed that the expression levels of the recombinant proteins reached a maximum between 24 and 36 h after infection (data not shown). Western blot analysis of extracts from infected cells using the T7.Tag antibody which reacts against the T7 gene 10 peptide tag detected the same polypeptides of 41.5 and 30 kDa (Fig. 1B, lanes 1 and 3), respectively. In addition, the T7.Tag antibody was able to immunoprecipitate the G11 polypeptides from labeled insect cells infected with the different G11 recombinant viruses (Fig. 1C). Analysis of the supernatants recovered after spinning down the immune complexes bound to the Protein A-Sepharose beads by SDS-PAGE revealed that all the G11 polypeptides had been successfully immunoprecipitated (data not shown).

G11 Has an Intrinsic Protein Kinase Activity—The G11-Z polypeptide was immunoprecipitated from detergent-solubilized insect cell extracts using the T7.Tag antibody. After washing the immunoprecipitates thoroughly with 150 mM NaCl, 1% (v/v) Triton X-100, 50 mM Tris-HCl (pH 7.4) they were subjected to an in vitro kinase assay where it was found that the immunoprecipitates catalyzed the transfer of [$\gamma$-32P]ATP to [gamma-casein (Fig. 2A, lanes 4 and 5)] was determined in a kinase assay, followed by SDS-PAGE and autoradiography. The ability of the immune complexes formed to phosphorylate protein $\alpha$-casein (A) and histone (B) was determined by the kinase assay, followed by SDS-PAGE and autoradiography. The ability of the immune complexes formed to phosphorylate protein $\alpha$-casein (A) and histone (B) was determined in a kinase assay, followed by SDS-PAGE and autoradiography. The ability of the immune complexes formed to phosphorylate protein $\alpha$-casein (A) and histone (B) was determined in a kinase assay, followed by SDS-PAGE and autoradiography.
the G11-Z polypeptide does not undergo autophosphorylation (data not shown).

In order to determine whether the removal of the N-terminal 110 amino acid residues of G11-Z had any effect on the efficiency of the kinase activity, immunoprecipitates of cell lysates infected with G11-Y and G11-Z were incubated in the kinase assay. SDS-PAGE analysis revealed that the incorporation of $^{32}$P into $\alpha$-casein and histone by each G11 immunoprecipitate was comparable (Fig. 2, A and B). This was confirmed in a time course experiment, but in this case in order to quantify the kinase activity the amount of radioactivity in the $\alpha$-casein band on the SDS-PAGE was determined in a PhosphorImager. Fig. 2C shows that the kinase activity in the G11-Y immunoprecipitate increased in a linear fashion with time and was comparable to the kinase activity in the G11-Z immunoprecipitate. This suggests that the segment of the G11 polypeptide between amino acids 111–368 most likely corresponds to the catalytic domain. A similar level of kinase activity (Fig. 2, A, lane 2, and C) was found to be associated with immunoprecipitates from cells infected with the G11-Y recombinant virus were immunoprecipitated with the T7.Tag antibody, the immune complexes were assayed for kinase activity in the presence of $\alpha$-casein for 20 min, and then different concentrations of FSBA (0.1, 0.5, 1, 5, 10, 20 mM, lanes 1–6, respectively) were added for another 20 min. D, the radioactivity in the $\alpha$-casein bands in the SDS-PAG shown in C was quantified using a PhosphorImager, and is compared with that obtained using immunoprecipitates from cells infected with wild type virus. M represents prestained molecular weight markers (in kDa). The arrowhead in A illustrates the location of the recombinant G11-Y polypeptide.

Several variables of the kinase reaction were examined. It was found that the optimal temperature of the reaction was 30 °C, and that the kinase activity started to be detectable after 10 min and increased in a linear fashion with time (Fig. 2C). The enzyme had an absolute requirement for an appropriate divalent cation with a rank order of Mn$^{2+}$ > Mg$^{2+}$ > Mn$^{2+}$ / Mg$^{2+}$ > Mn$^{2+}$ / Ca$^{2+}$, and the cation was more effective at a concentration of 20 mM than at a concentration of 10 mM (data not shown). Carrying out the reaction in the presence of 20 mM CaCl$_2$ did not lead to any detectable level of $^{32}$P incorporation.

**G11 Phosphorylates Ser/Thr Residues of Acceptor Proteins**

The identity of the amino acids serving as phosphate acceptors in $\alpha$-casein and histone during the kinase reaction using immunoprecipitates containing the G11-Y recombinant protein was determined by two-dimensional electrophoretic analysis of partially acid-hydrolyzed $^{32}$P-labeled products. As shown in
Fig. 3A the amino acid acceptors in α-casein were Ser and Thr, and in histone it was Ser only (Fig. 3B). No tyrosine phosphorylation was observed suggesting that G11 is a Ser/Thr protein kinase.

Affinity Labeling of G11 with FSBA—Selective labeling of protein kinases can be carried out with the reactive ATP analogue FSBA which has been shown to react with an invariant lysine residue present within the ATP-binding site of the protein kinase superfamily (18). To test this, extracts from insect cells infected with either the G11-Y recombinant virus or with the wild type virus were incubated in the kinase buffer with 1 mM FSBA for different times, in the presence or absence of 1 mM ATP. The G11-Y polypeptide was immunoprecipitated with the T7.Tag antibody and the labeling of G11-Y with FSBA was detected by immunoblotting with affinity-purified anti-FSBA antibodies. The Ser/Thr casein kinase II was used as a control to monitor the successful labeling of protein kinases with FSBA (Fig. 4, A and B, lane 5). Fig. 4A shows a ladder of FSBA reacted proteins, all of which are detected in the sample incubated in the presence (lanes 1 and 2) or absence (lanes 3 and 4) of 1 mM ATP. There is only one protein of 30 kDa, corresponding to the expected size of G11-Y, which is present in the sample incubated in the absence of 1 mM ATP (Fig. 4A, lane 4), but which is absent in the sample incubated in the presence of 1 mM ATP (Fig. 4A, lane 2). The 30-kDa protein is not detected in the wild type samples whether in the presence (Fig. 4B, lanes 1 and 2) or absence (Fig. 4B, lanes 3 and 4) of 1 mM ATP. These results showed that FSBA covalently binds to the 30-kDa G11-Y polypeptide and that this binding can be blocked by ATP, suggesting that G11-Y is capable of binding ATP. To complement the results stated above the inhibition of ATP labeling by FSBA was investigated. The results of kinase assays using the G11-Y immunoprecipitates shown in Fig. 4C revealed that the kinase activity decreased as the concentration of FSBA increased, and was completely inhibited by 5 mM FSBA (Fig. 4D).

Enzyme Activity and Localization of G11 in COS-7 Cells—In order to further characterize the G11-Z protein and determine its cellular localization the entire protein-coding region of G11-Z including the T7.Tag epitope at the N-terminal end was inserted into the mammalian expression vector pcDNA3 and expressed in COS-7 cells. A unique band of 41.5 kDa corresponding to G11-Z was detected with the T7.Tag antibody on Western blot analysis of lysates from G11-Z transfected cells (Fig. 5A, lanes 1 and 2) which was not present in lysates from cells transfected with a pcDNA3-CAT construct encoding bacterial chloramphenicol acetyltransferase (CAT) (Fig. 5A, lane 3). To identify the G11-Z enzymatic activity in transfected COS-7 cells, the G11-Z polypeptide was immunoprecipitated from cells transfected with the pcDNA3-G11Z construct using the T7.Tag antibody. As shown in Fig. 5B (lanes 1 and 2) the G11-Z immunoprecipitates were able to catalyze the transfer of $^{32}$P from [γ-$^{32}$P]ATP to α-casein. No kinase activity was detectable in immunoprecipitates from COS-7 cells transiently expressing CAT (Fig. 5B, lane 3) or the 43-kDa sialidase (13) containing the same peptide tag (data not shown).

To provide further evidence that G11 is a protein kinase, mutants were constructed by introducing a point mutation resulting in the conversion of a lysine to a proline at amino acids 300, 315, and 317 in the catalytic domain of the enzyme, respectively. The mutant cDNAs and the G11-Z cDNA without the T7 gene 10 peptide tag were inserted into pBK-CMV. To identify the enzymatic activity of these G11 mutants, the G11–300R, G11–315R, G11–317R, and the wild type polypeptides were immunoprecipitated from transfected COS-7 cells using the anti-G11 monoclonal antibody and subjected to a kinase assay. As shown in Fig. 6 (lane 4) when the lysine residue at amino acid 317 was converted to a proline the kinase activity was completely ablated. Furthermore, the conversion of the lysine residues at amino acids 300 and 315 to a proline resulted in a dramatic decrease in the levels of activity (lanes 2 and 3, respectively), whereas the wild type (lane 5) showed the expected levels of kinase activity.

To determine the subcellular localization of the G11-Z protein, COS-7 cells were transiently transfected with the tagged G11-Z coding sequence or the CAT coding sequence, then stained with the T7.Tag antibody. Cells expressing the tagged G11-Z polypeptide showed an intense nuclear staining (Fig. 7, panel C). An identical pattern of nuclear staining was seen in cells when the control antibody to nuclear splicosomes was used (Fig. 7, panel B). This intense nuclear staining was not seen when COS-7 cells were transfected with the CAT construct and stained with the T7.Tag antibody (Fig. 7, panel D), or with a construct encoding the 43-kDa sialidase containing the same peptide tag which was visualized in the endoplasmic reticulum (13). In addition, a construct encoding the cytoplasmic protein HSP70-Hom with the same epitope tag at the N
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FIG. 7. Localization of the G11-Z polypeptide was analyzed by immunofluorescence. COS-7 cells growing on coverslips were transfected with pcDNA3-G11Z (C) or pcDNA3-CAT (A, B, and D). The cells were incubated with the antibody to α-tubulin (A), with the antibody to nuclear splicosomes (B), or with the T7.Tag antibody (C and D). The scale bar represents 20 μm.

Phe-Ser, does not include the third Gly residue either (23). In all known protein kinases there is a highly conserved lysine residue which is thought to be involved in the binding of ATP and that lies 14 to 23 residues downstream of the last conserved glycine in the consensus sequence Gly-X-Gly-X-Gly (21). Lysine residues at amino acids 300, 315, and 317 of the G11-Z sequence are therefore possible candidates. In this study we show that the conversion of the lysine residue at amino acid 317 to a proline completely ablated the enzyme activity of G11 while the kinase activity of G11–300R and G11–315R was decreased dramatically. This is consistent with our observation that G11 expressed in insect cells is capable of being covalently modified with FSBA and that this modification can be inhibited by ATP. These results provide direct evidence that the G11 protein possesses a phosphotransferase activity.

Sequence analysis of reverse transcribed RNA from a number of different cell lines has shown the presence of sequences that contained a 12-bp insertion (7) between nucleotides 790 and 791 of the G11-Z cDNA sequence. This 4-amino acid difference is quite important as it results in the expression of G11 polypeptides which contain or lack the sequence Val-Cys-Asp-Cys in the kinase domain. However, a similar level of kinase activity was found to be associated with immunoprecipitates from cells infected with the G11-Y(ΔCΔD) construct compared with cells infected with the G11-Y construct suggesting that the absence of these 4 amino acids does not significantly affect the kinase activity associated with the G11 protein. The region that is not included in the catalytic domain of protein kinases plays a major role in the regulation of the kinase activity. It is interesting to note that the N-terminal end of the G11 protein contains potential sites of phosphorylation at Ser/Thr and Tyr residues (7), suggesting that its function may be modulated through phosphorylation by other protein kinases.

Sequence analysis of G11-Z showed the presence of a specific bipartite nuclear target sequence (24, 25) between amino acids 114 and 131 (KRHHLIPPETFGVKRRRRK). The functional consequence of the presence of this sequence in G11-Z was experimentally supported when COS-7 cells transfected with the G11-Z coding sequence revealed intense staining of the nucleus. The similarity of the G11-Z protein with nuclear proteins was further indicated by the presence of one putative kinase C phosphorylation site (amino acid 112) near the nuclear target sequence. Phosphorylation near nuclear target sequences has been proposed to accelerate the rate of nuclear uptake and to play a role in cell cycle dependent control of nuclear entry of oncogene products and of transcription factors (24).

Protein kinases control a wide range of cellular events and in many cases individual enzymes are involved in the regulation of multiple different processes. Therefore, it is important that these enzymes select the correct substrates at the correct time to coordinate the complex organization of cellular events. One factor involved in substrate selection is substrate structure (26). However, studies have also shown that another major factor determining target specificity may be the restricted subcellular distribution of kinase activities, through the subcellular compartmentalization of the enzymes or their activators (26). This suggests that the substrate of G11-Z is located in the nucleus and that through phosphorylation G11-Z might be involved in the regulation of transcription in the nucleus.

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