Human Vaccinia-related Kinase 1 (VRK1) Activates the ATF2 Transcriptional Activity by Novel Phosphorylation on Thr-73 and Ser-62 and Cooperates with JNK

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In the human kinome, vaccinia-related kinase-1 (VRK1) is a new Ser-Thr kinase associated with proliferating tissues. VRK1 colocalizes with ATF2 in the nucleus and can form a stable complex. We have studied the phosphorylation of the transcription factor ATF2, which regulates gene expression by forming dimers with proteins with basic region-leucine zipper domains and recognizing cAMP-response element or AP1 sequences implicated in cellular responses to stress. VRK1 phosphorylates ATF2 mainly on Thr-73, stabilizing the ATF2 protein and increasing its intracellular level. Mutagenesis studies showed that Thr-73 and Ser-62 are implicated in ATF2 transcriptional activation by VRK1 detected in a functional assay based on ATF2 dimerization. VRK1 can activate the collagenase gene promoter that is regulated by ATF2 in a dose-dependent manner. Loss of kinase activity (K179E mutant) or the T73A substitution in ATF2 prevents both its accumulation and activation of transcription. VRK1 and JNK, which phosphorylates ATF2 in Thr-69 and Thr-71, have an additive effect on ATF2-dependent transcription at suboptimal doses. Therefore, two groups of amino acids in the ATF2 amino-terminal region can integrate different cellular signals mediated by at least five different kinases. VRK1 is an element of a novel signaling pathway that regulates gene transcription.

Phosphorylation of transcription factors is a major regulatory mechanism of gene expression in the cellular response to many types of stress (1). Some of these transcription factors belong to the activating transcription factor (ATF)1/cAMP-response element-binding (CREB) protein family of basic region-leucine zipper (b-ZIP) proteins (2), which are involved in the response to stress, such as viral infections (3, 4). Furthermore, these proteins are regulated by phosphorylation in their amino-terminal region (5).

The ATF protein family is composed of several subgroups based on their amino acid similarity and includes CREB, ATF2, CRE-BP1, ATF3, ATF4, ATF6, and B-ATF (6). These proteins have two domains. One is a leucine zipper (7) that permits their dimerization either with themselves or with members of other protein families that also contain b-ZIP sequences, thus forming either homo- or heterodimers (2, 6), such as those of the CCAAT/enhancer-binding protein (C/EBP) and AP1 families, which include c-Jun and c-Fos (2). The homo- or heterodimeric complexes confer a large flexibility to these factors by modulation of their DNA binding specificity (8) and thus increase their regulatory potential. These proteins have a DNA binding domain that recognizes a core sequence located in the promoters of some viral genes, such as adenovirus E1A (9). This core DNA sequence also mediates the cell response to fluctuations in the intracellular levels of cyclic AMP (5) and in intracellular free calcium (10). The CRE (cAMP-response element) proteins recognize a specific core sequence on a variety of gene promoters that are likely to be context-dependent (5, 11, 12), such as in the insulin gene (13). The specificity of ATF2 can also be affected by interactions with viral proteins such as hepatitis B X protein (14, 15). ATF2 also contributes to the regulation of genes implicated in cell growth, differentiation, immune response, and response to stress. Some of these genes are c-Jun (16), collagenase (17), cyclin A (18), and tumor necrosis factor-α (19), among others.

In the absence of stimulation, ATF2 has a relatively low transactivation role because there is an intramolecular inhibitory interaction in which the DNA binding domain is folded and binds to the amino-terminal transactivation domain (20–22). This conformation can be relieved by phosphorylation (23). Phosphorylation in the NH2-terminal domain of ATF2 increases its transcriptional activity (3, 4). These proteins are regulated in response to stress kinases such as the c-Jun NH2-terminal kinase (JNK) (24, 25) or p38 (23, 26). JNK and p38 mitogen-activated protein kinase phosphorylate ATF2 at Thr-69 and Thr-71, stimulating its transactivation potential (27–29). Phosphorylation at these residues also stabilizes ATF2 (23) and modulates its acetyltransferase activity (30). More recently a novel phosphorylation of ATF2 has been detected at
Thr-73, in combination with Thr-69 and Thr-71 (13). This phosphorylation is mediated by the Ca2+/calmodulin-dependent protein kinase IV (CaM kinase IV), which is required for the ATF2-dependent positive regulation of the human insulin gene (13). Also protein kinase A phosphorylates ATF2 at Ser-62 in response to cAMP (31).

In the human kinome, the VRK gene family represents a novel branch from the lineage that led to the group of casein kinases (32). The human VRK protein was originally identified by their homology with the BIRC kinase of vaccinia virus, an early protein of the vaccinia virus; this virus is responsible for the induction of the immune response (33–35). The ortholog genes in yeast, Hhr25 in Saccharomyces cerevisiae and Hhp1 in Schizosaccharomyces pombe, have been implicated in responses to DNA damage (36). However, such a role has not yet been identified for the human VRK proteins. The VRK kinase family is composed of three proteins, of which only VRK1 and VRK2 have been shown to be active, despite the relatively weak conservation of its kinase domain (37, 38). The three VRK proteins differ in their putative regulatory domain, which is located in the carboxyl terminus for VRK1 and -2 and in the amino terminus for VRK3 (39). VRK1 is highly expressed in proliferating tissues, such as tumor cell lines (40), and during the hematopoietic proliferative phase in murine embryo midgestation (41). VRK1 is a novel nuclear kinase, detected in both murine and human cells, that regulates p53 by phosphorylation on Thr-18 (37, 38), a residue essential for the interaction of p53 with p53-binding protein 1 (42). This effect of VRK1 suggests that it might play a role in mechanisms controlling cellular responses to stress and DNA damage. Because of the relationship of p53 to stress signals, we decided to determine whether this kinase is also able to phosphorylate other substrates that are known to be phosphorylated in response to stress signals. In this work we report the phosphorylation of the transcription factor ATF2 by a new kinase, VRK1. This phosphorylation takes place in novel residues within the amino terminus of ATF2 and results in its stabilization and increased transcriptional activity. Furthermore, the ATF2 phosphorylation by VRK1 can cooperate with the phosphorylation mediated by JNK.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Fusion Proteins**—Bacterial expression construct pGEX-ATF2 (1–109) was a gift from R. Green (University of Massachusetts) (45), and GST-VRK1 fusion protein was described previously (37). Mammalian expression plasmid pHA-ATF2 with full-length cDNA was a gift from K. Yokoyama (Tsukuba, Japan) (30). The reporter plasmid used in all of the transient transfection assays, 5× GAL4-Luc reporter, and HA-JNK1 constructions were a gift from S. Gutkind, and pSG-424-ATF2 was a gift from K. Yokoyama (Tsukuba, Japan) (30). The reporter plasmid pSG-424-ATF2 to be used in transcription assays. All mutations were confirmed by DNA sequencing.

**Cell Lines and Transfections**—293T human embryonic kidney and HeLa human cervical adenocarcinoma cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin at 37°C in a 5% CO2 atmosphere. NIH3T3 mouse fibroblasts were grown on uncoated glass coverslips introduced into 100-mm plates grown on uncoated glass coverslips introduced into 100-mm plates. 30 h posttransfection using JetPEI (Polytransfection, Illkirch, France) according to the manufacturer’s recommendations. The total number of DNA within the experiments was kept constant by adding the respective empty vector plasmid DNA to the transfection mixtures.

**Immune Complex Kinase Assays and Western Blots**—Cells were washed twice with ice-cold phosphate-buffered saline (PBS), harvested with a rubber policeman, and centrifuged at 4°C. Cell pellets were resuspended in ice-cold lysis buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 1.5 mM MgCl2, 10 mM NaF, 10% glycerol, 4 mM EDTA, pH 7.4, plus 1% Triton X-100, 0.1% SDS) and protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride), incubated on ice for 30 min, and precleared by centrifugation at 17,000 × g for 20 min at 4°C. Extracts were immunoprecipitated with an anti-HA antibody (Covance, Berkeley, CA) or with an anti-VRK1 rabbit polyclonal antibody made against the GST-VRK1 fusion protein. Immune complexes were recovered with γ-Disp G-Sepharose (Amersham Biosciences). Beads were sequentially washed five times with lysis buffer, once with 10 μM Tris-HCl at pH 7.5, and once with kinase buffer. The phosphorylation reaction was performed as described previously (37). Whole cell extracts were collected 48 h posttransfection in lysis buffer, and 25–50 μg were analyzed for the expression of VRK1 and -2 and ATF2-full-length and mutants proteins with anti-HA antibody. The anti-HA and anti-AU monoclonal antibodies (IgG1 isotype) were from Covance. Western blots were performed and developed by chemiluminescence techniques using the ECL detection system and horseradish peroxidase-conjugated anti-mouse antibody (Amersham Biosciences).
with a secondary antibody, either anti-mouse secondary antibody coupled to Cy2 (Sigma) (1:200 dilution) or anti-rabbit secondary antibody coupled to Cy3 (Sigma) (1:200 dilution). Fluorescence images were captured with a Zeiss LSM 510 confocal microscope. Fluorochromes were excited using an 18Ar laser (488-nm excitation wavelength) for Cy2 and a 1He/1Ne laser (543-nm excitation wavelength) for Cy3.

**Transcriptional Assays**—Reporter gene assays were performed with the Dual-Luciferase reporter assay system (Promega) at 36–48 h after transfection. The assay is based on the use of pSG-424-ATF2 (1–109) constructs with a GAL4 DNA binding domain, or its mutants and a p5×GAL4-Luc reporter were cotransfected in each sample in combination with phRL-tk Renilla luciferase as an internal control for transfection efficiency. The total amount of DNA within the experiments was kept constant by adding the respective empty vector plasmid DNA to the transfection mixtures. The activity of the reporter luciferase was expressed relative to the activity in control vector-transfected cells. Similar results were obtained in at least three different experiments. All results were analyzed by comparing with the control (unpaired Student's t test), and shown in the figures is the mean ± S.D. of independent triplicate cultures as well as the p value.

**RESULTS**

**ATF2 and VRK1 Colocalize in the Nucleus**—The phosphorylation by the human VRK1 protein of p53 (37) suggested that it might also phosphorylate other transcription factors that participate in the cellular response to stress mediated by other kinases, such as JNK and p38. To determine whether there was a colocalization of the transcription factor ATF2 and the VRK1 protein, HeLa cells were transfected with pHA-ATF2 and pVRK1-Myc, and their subcellular location was determined with an antibody specific for the HA epitope. The transfected ATF2 is located in the nucleus of transfected cells and shares the same compartment as the VRK1 protein (Fig. 1A). Interestingly, the cells that have taken up both plasmids appear to have a higher level of the ATF2 protein that was detected as a more intense green fluorescence signal.

Smaller fractions of the two proteins overlap as nuclear signals, detected as yellow, indicating that in the nucleus VRK1 and ATF2 have proximal locations. To show that in the nucleus some of the VRK1 and ATF2 molecules colocalize, an immunoprecipitation and immunoblot study was performed. HeLa cells were cotransfected with pHA-ATF2 and pVRK1-Myc plasmids. Extracts from transfected cells were immunoprecipitated with an anti-HA antibody (detects HA-ATF2) followed by an immunoblot analysis against either HA-ATF2 or VRK1. The result is shown in Fig. 1B. In the ATF2 immunoprecipitate the VRK1 protein is clearly detected (Fig. 1B, right panel) suggesting that at least a proportion of the molecules do form a complex in the nucleus. The signal for ATF2 is weaker (Fig. 1B, left panel) because in the immunoblot the same antibody was used for immunoprecipitation and blotting; thus the secondary antibody used in the detection system also recognizes the immunoglobulin chains present in the immunoprecipitate and thus competes with the specific signal. Therefore, to rule out the potential contribution of nonspecific binding to the antibody used in the immunoprecipitation, a control experiment was performed in which extracts from cells transfected with VRK1 and ATF2 constructs were also immunoprecipitated with another monoclonal antibody of the same isotype (IgG1) against the AU5 epitope that is not present in these cells. In this case VRK1 is detected only when ATF2 was immunoprecipitated but not in the control with the nonspecific antibody (Fig. 1C).

**Phosphorylation of ATF2 by VRK1**—To determine whether VRK1 was able to phosphorylate the human ATF2 protein, an in vitro kinase assay was performed. The cloned human VRK1 fused to GST was expressed as a GST-VRK1 fusion protein (37) and tested for the phosphorylation of ATF2 in vitro using as substrate a GST-ATF2 fusion protein containing residues 1–109 (Fig. 2A). The phosphorylation takes place within the ATF2 ami-

**FIG. 1.** Colocalization and interaction of VRK1 and ATF2 in the cell nucleus. A, HeLa cells were transfected with pVRK1-Myc and pHA-ATF2. VRK1 was detected with a specific polyclonal antibody for the Myc epitope (red), and ATF2 was detected with an anti-HA monoclonal antibody (green). Dapi, 4',6-diamidino-2-phenylindole. B, HeLa cells were transfected with an empty vector as a control (1) or with pVRK1-Myc and pHA-ATF2 (2). The whole cell extract contains both proteins as shown in the top immunoblot. The extract was immunoprecipitated (IP) with an anti-HA antibody and divided in two. One half was immunoblotted (IB) with the same antibody (anti-HA) where ATF2 is detected (lower left blot) as well as the immunoglobulin bands because the antibody combination used reduces the specific signal because of competition. The other half of the immunoprecipitate was immunoblotted with a polyclonal antibody specific for human VRK1 (lower right blot). C, specificity of the VRK1 band brought down by anti-HA-ATF2. The cellular extract from cells transfected with HA-ATF2 and VRK1 (similar to lane 2 in B) was immunoprecipitated with a monoclonal antibody (IgG1) against an epitope (AU5) not present in these cells and with the anti-HA-ATF2 monoclonal antibody (IgG1) followed by detection with an anti-VRK1 antibody. VRK1 was detected only when ATF2 was immunoprecipitated.
indicated at the electrophoresis and thin layer chromatography. The specific mutant is the amino-terminal region. Toward this aim 293T cells were transfected with increasing amounts of pHA-VRK1 or its inactive mutant (K179E) and cotransfected with a fixed amount of pHA-ATF2. From these experiments it was concluded that the endogenous human VRK1 also can phosphorylate ATF2 in its amino-terminal region.

**VRK1 Phosphorylates ATF2 on Thr-73 and Ser-62**—To identify the specific residues within the ATF2 molecule that are phosphorylated by VRK1, all the potential Ser and Thr targets were mutated individually. However, if more than one residue is phosphorylated, polyacrylamide gels will not detect the complete loss of the phosphorylated protein. The ATF2 mutants made contained the following individual substitutions: S9A, S20I, T28A, T37A, T52A, S62I, T69A, T71A, T73A, and S90I. In single dimension polyacrylamide gels, wild type ATF2 and all of the ATF2 mutants were phosphorylated. Although in some substitutions, such as T73A, a decrease in the incorporation of radioactivity was detected, it appeared that more than one residue was phosphorylated (not shown). To ascertain that more than one residue was phosphorylated, we next performed a phosphoamino acid analysis, and both a phosphoserine and a phosphothreonine were detected, confirming that indeed there are two residues phosphorylated in the ATF2 molecule by VRK1 (not shown).

Because at least two different residues are phosphorylated by VRK1, we performed by digestion with trypsin a phosphopeptide map of the different GST-ATF2 proteins, wild type or with the different amino acid substitutions. Four major spots were detected, two weak and two relatively stronger. These analyses clearly identified residues Ser-62 and Thr-73 as the major targets for VRK1 (Fig. 2C). In each of these substitutions, S62I and T73A, two phosphopeptide spots disappeared. The spots corresponding to the Thr-73 position accounted for most of the radioactivity incorporated. Also in the chromatoplaques and after long exposures, there were many weak phosphorylated peptides, probably representing a marginal activity of the kinase because individually they were at least 1 order of magnitude less intense; however, they appear as single spots in the phosphoamino acid study. The substitutions of the residues targeted by JNK and p38, T69A and T71A, were not phosphorylated by VRK1 (Fig. 2C). When a T71A or T73A substitution was made, a change must have occurred in the conformation of the protein because there was a strongly phosphorylated new phosphopeptide, which was detected as a fast moving spot (Fig. 2C), suggesting that these substitutions induce a change in conformation that makes new residues accessible to the kinase.

**VRK1 Induces an Accumulation of ATF2 in the Cell**—The phosphorylation of ATF2 by other kinases, such as JNK, resulted in an increase in its stability and consequently in higher intracellular levels of ATF2 because the phosphorylated molecules can dimerize, preventing their binding to ubiquitin ligase (23). Therefore, we tested whether VRK1 could also induce an accumulation of ATF2 as a consequence of its phosphorylation in the amino-terminal region. Toward this aim 293T cells were transfected with increasing amounts of pHA-VRK1 or its inactive mutant (K179E) and cotransfected with a fixed amount of pHA-ATF2. The levels of ATF2 and VRK1 proteins were determined by immunoblots. The ATF2 protein, detected with an anti-HA antibody, was accumulated as the amount of VRK1 protein increased (Fig. 3A), but there was no accumulation when the inactive VRK1 mutant (K179E) was used (Fig. 3B). The quantification of the accumulation effect induced by the active or inactive kinase is shown below the Western blots in Fig. 3. To confirm that the effect is specific, a construct expressing the EGFP protein under the same promoter of CMV was also transfected, and the levels of EGFP were determined by Western blot. The levels of EGFP were not affected by increasing the amount of transfected VRK1 (Fig. 3A). Also, the control protein, β-actin, was not affected by changes in the VRK1 protein.

The accumulation of the ATF2 molecule by VRK1 requires the phosphorylation of Thr-73. When the ATF2 T73A mutant was used in the assays, there was no accumulation of ATF2 inducible by VRK1 (Fig. 3C). Also the ATF2 double mutant...
VRK1 was not able to accumulate in response to VRK1 (T73A/S62I).

**VRK1 Activates ATF2-dependent Transcription**—Because ATF2 is a transcription factor, the next aim was to determine whether VRK1 could also activate the ATF2-dependent transcriptional activity. This assay is dependent on the dimerization of phosphorylated ATF2 because it is based on the use of a construct containing the ATF2 amino terminus fused to the GAL4 DNA binding domain. Transcriptional activity was determined using a GAL4-Luc reporter system, and the activation requires the binding of a dimer to the GAL4 DNA binding site. The dimer is formed following phosphorylation. First a dose-response assay was performed in 293T cells. As the amount of transfected VRK1 was increased as detected by Western blot, the transcriptional activity dependent on ATF2 likewise increased (not shown). Next it was determined through experiments using luciferase reporter constructs whether phosphorylation of ATF2 in residues Ser-62 and Thr-73 has any role in the modulation of ATF2 transcriptional activity. These experiments were performed in 293T cells transfected with either pHA-VRK1 or pHA-JNK1 as a positive control. As substrates, constructs with the transactivation amino terminus of ATF2 (residues 1–109) were used. The increase in activity induced by VRK1 was similar to that induced by JNK (Fig. 4A). In this ATF2 moiety-specific amino acid residues were substituted to determine their consequences on the transcriptional activity of the reporter luciferase gene. The individual ATF2 substitutions S62I or T73A resulted in the loss of transcriptional activation by VRK1 (Fig. 4A). The activation of ATF2-dependent transcriptional activity by VRK1 was also detected using NIH3T3 fibroblasts transfected with either pHA-VRK1 or pHA-JNK1, as a positive control, to show that the effect is more general and not restricted to a single cell type (Fig. 4B).

**VRK1 Can Cooperate with JNK in the Activation of ATF2**—Because JNK and VRK1 can phosphorylate ATF2 in different residues within the same region of the protein, it is possible that under conditions in which neither of the two has a maximal effect their activation could be cooperative and that the kinase effect could be additive. In an attempt to reproduce this potential cooperation 293T cells were transfected using subop-

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**Fig. 3. Accumulation of the ATF2 protein induced by VRK1.** A, VRK1 can induce an accumulation of ATF2 that is dependent on the amount of VRK1. 293T cells were transfected with a fixed amount of pHA-ATF2 (1 μg) and varying amounts of pHA-VRK1 (indicated in the figure). A control experiment was performed transfecting the cell with another gene under the same CMV promoter; for this purpose a pCMV-EGFP construct was used, and the protein was detected with an anti-EGFP antibody. B, similar experiment using the inactive pHA-VRK1 (K179E) protein. The quantification of the accumulation induced by the active (black bars) and inactive (gray bars) kinases is shown below the Western blots. C, lack of accumulation of ATF2 if the Thr-73 residue was changed to alanine (T73A). D, lack of accumulation of ATF2 containing a double substitution. Ser-62 was changed to isoleucine (S62I), and Thr-73 residue was changed to alanine (T73A).
timal doses of kinases, either a fixed amount, 0.4 μg of either JNK1 or VRK1, or a combination of both (0.4 μg of each). As shown in Fig. 6, the mixture of the two kinases appeared to have an additive effect, similar to that of each individual kinase, at an equivalent dose (0.8 μg), which targets a unique pair of residues rather than their combination. This observation suggests that ATF2 can potentially respond to two different signals.

**DISCUSSION**

VRK1 gene expression is apparently ubiquitous, being present in all cell types studied. However, there are very large differences when the proliferation state of the cell is taken into consideration. VRK1 appears to be overexpressed in tumor cells as well as in highly proliferating cells, such as regenerating liver (40), and in proliferating phases during murine embryonic development (41). All these data suggest that VRK1 might participate in the cellular protection mechanisms needed at a time when the possibility of accumulating genetic damage is the highest. In this context it is important to note that VRK1 phosphorylates p53, enhancing the protective role played by this tumor suppressor protein, a key element in the responses to different types of cellular stress (37). ATF2 is a transcription factor that participates in the responses mediated by cyclic AMP but is also involved in some stress responses. ATF2 colocalizes with VRK1 kinase in the nucleus, but most importantly the two proteins can form in the nucleus a stable complex, VRK1-ATF2, that can be immunoprecipitated. The complex, however, represents a small subpopulation of the two proteins present in the nucleus, and at this moment its significance is unknown. Because VRK1 has an activity related to casein kinase I, its interactions with target proteins are expected to be transient and not very stable.

The ATF2 residues phosphorylated by VRK1 differ from those phosphorylated by stress kinases. The stress kinases JNK and p38 phosphorylate Thr-69 and Thr-71 in the ATF2 amino terminus. However, the ATF2 phosphorylation sites by VRK1 are located in the same region of the ATF2 molecule, mainly in Thr-73 and also in Ser-62, as shown by the incorporation of most
of the radioactivity in Thr-73. The consequence of this ATF2 phosphorylation is an increase in its intracellular level, detected in transfected cells by fluorescence as well as by Western blot. Although these two residues have so far received little attention, they are known to be phosphorylated by other kinases. Ser-62 is phosphorylated by protein kinase A, a cAMP-dependent kinase (31), and Thr-73 is also phosphorylated by CaM kinase IV, a calcium-dependent kinase (13). The consequence of VRK1 overexpression is the accumulation of ATF2 protein. This accumulation was lost if the cells were transfected with a catalytically inactive VRK1 kinase (K179E) or if the target residue in ATF2 was changed with the T73A substitution. This stabilization may be explained as a consequence of the unfolding of the phosphorylated ATF2 molecule that will favor its homo- or heterodimerization and will prevent the interaction of its amino terminus with ubiquitin ligase (23, 46, 47). This can explain why transfection with increasing amounts of VRK1 leads to an accumulation of ATF2, suggesting that the fraction of phosphorylated ATF2 is large and therefore is not degraded.

The consequence of accumulating phosphorylated ATF2 is an increase in its dimerization potential and consequently in its transcriptional activity. This has been demonstrated using two systems. One is based on the use of the transactivation domain of ATF2 fused to a GAL4 DNA binding domain. This assay reflects the consequence of homodimerization of the ATF2 factor. In the second system a specific gene promoter, the collagenase promoter known to respond to ATF2 through a known AP1 binding site, is used (17). This second system confirmed the activation of ATF2 transcription in the presence of VRK1. The magnitude of the activation is not very large, 2–3-fold, but it is known that ATF2 activation by itself is not very large and that it is usually enhanced by cooperation with other transcription factors present on the target promoter. The targeting of different residues on the ATF2 molecule permits the possibility of different signals at suboptimal doses cooperating in the activation of ATF2, as shown when transfected cells with both VRK1 and JNK.

The regulation of ATF2 by phosphorylation is very complex because this is performed by many different kinases responding to very heterogeneous types of stimulation (5, 12). Among these kinases are protein kinase A, CaM kinase IV, JNK, and p38, to which now VRK1 has to be added, as shown in the diagram illustrating this regulatory complexity (Fig. 7). Curiously, these phosphorylations have not been studied simultaneously under physiological conditions, but this type of study is likely to shed light on many apparently different effects. Furthermore, it is not known whether phosphorylations of different residues on the ATF2 molecule could affect the dimerization properties in such a way that phosphorylations in specific residues might favor binding preferentially to some partners but not to others (48). If that turns out to be the case, it will represent an additional mechanism by which the selection of promoters is performed because each dimer combination may have different promoter specificity. Thus a dual effect is obtained affecting dimer combination and promoter selection.

Different phosphorylation sites raise the issue of potential kinase cooperation. ATF2 is likely to integrate many types of cellular signals that reach the transcription factor by different kinases (Fig. 7), some of which do not phosphorylate the same residues, although all phosphorylatable amino acids are within the same region of the ATF2 amino terminus. In this report we have shown that phosphorylation by two kinases, VRK1 and JNK, targeting different amino acids can have an additive effect in the activation of transcription. This activation, depending on its level, may cooperate with other signals if all of them are acting at a suboptimal dose or may be exclusive if any of them reaches a maximum effect. It can be concluded that VRK1 represents the beginning of a new signaling pathway of which additional elements remain to be identified.
VRK1 Phosphorylation of ATF2 on Thr-73

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