Mutations That Increase Acidity Enhance the Transcriptional Activity of the Glutamine-rich Activation Domain in Stage-specific Activator Protein*

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Sea urchin stage-specific activator protein (SSAP) activates transcription of the late H1 gene at the mid-blastula stage of development. Its C-terminal 202 amino acids form a potent glycine/glutamine rich activation domain (GQ domain) that can transactivate reporter genes to levels 5-fold higher than VP16 in several mammalian cell lines. We observed that, unlike other glutamine-rich activation domains, the GQ domain activates transcription to moderate levels in yeast. We utilized this activity to screen in yeast for intragenic mutations that enhance or inhibit the transcriptional activity of the GQ domain. We identified 37 loss of function and 23 gain of function mutants. Most gain of function mutations increased the acidity of the domain. The most frequently isolated mutations conferred enhanced transcriptional activity when assayed in mammalian cells. These mutations also enhance the ability of SSAP to up-regulate the late H1 promoter in sea urchin embryos. We conclude that the GQ domain fundamentally differs from other glutamine-rich activators and may share some properties of acidic activators. The ability of acidity to enhance SSAP-mediated transcription may reflect a mechanism by which phosphorylation of SSAP activates late H1 gene transcription during embryogenesis.

Correct spatial and temporal control of gene expression requires the organized recruitment of dozens of polypeptides to the promoters of individual genes. Transcriptional activators make up one brigade of this molecular army (1). These proteins typically contain a transcriptional activation domain, separate from the DNA-binding domain, which confers upon it the ability to up-regulate its target genes. A functionally accurate grouping of this brigade into divisions and subdivisions has escaped investigators for years. The lack of discrete, conserved structural elements between different activation domains has bred instead a loose classification scheme based upon overall amino acid content. The major families include acidic activators, such as Gal4 (2) and VP16 (3), glutamine-rich activators, such as Sp1 (4), proline-rich activators, such as CTF1 (5), and basic activators, such as that in T3Rb (6). Most evidence suggests that activation domains exist under physiological conditions as unstructured, sticky polypeptide chains (Ref. 7; reviewed in Ref. 1). Their job entails establishing protein-protein interactions with components of the basal transcription machinery, either directly or via coactivators and mediator proteins, and thereby stimulating any of several steps in the complicated pathways of transcription initiation or elongation (reviewed in Ref. 8).

At the level of amino acid sequence, the motifs or characteristics that confer these biological activities upon a polypeptide remain elusive. Initial mutagenesis studies on the acidic Gal4 domain implicated acidity as an overall biochemical property that confers activation potential upon a polypeptide (9, 10). However, subsequent studies suggested that other, nonacidic residues in acidic domains may contribute to the induction of secondary structure upon protein binding. In particular, the periodic spacing of hydrophobic residues in VP16 (11, 12) and other acidic domains (13, 14) led to the hypothesis that binding of an activator to its partner may induce an α-helical structure that, in turn, stabilizes the interaction. For at least one acidic activator, this has been shown to be the case: the acidic domain of CREB assumes an α-helical conformation upon binding to its in vivo interacting partner CBP (15). A similar controversy exists with respect to the glutamine-rich activators. Whereas individual glutamine residues themselves appear to lack critical significance, periodic spacing of hydrophobic residues seems to be important for activation mediated by these proteins as well (16). Some activities of transcription factors have even been mimicked by synthetic synthesis of polypeptide chains (17), suggesting that perhaps the only property necessary for a domain to function in activated transcription is the ability to bind to a general transcription factor (8). In all cases, however, the attributes that distinguish an activation domain from a random polypeptide remain to be determined.

Stage-specific activator protein (SSAP)1 is a sea urchin transcription factor responsible for the developmental activation of the late H1 histone gene at the mid-blastula stage of embryogenesis (18). In vivo, this protein binds as a dimer to three sites in a stage-specific enhancer 300 bases upstream of the late H1 transcription initiation site (19). Functional analysis revealed that the SSAP mRNA encodes a 41-kDa protein composed of two separable domains (20, 46). The N-terminal 180 amino acids make up the sequence-specific DNA-binding domain of SSAP and are sufficient to target the protein to its native binding site. The C-terminal 202 amino acids form a glycine-glutamine rich domain, the GQ domain, which shares homology with the glutamine-rich activation domains of the EWS and TSL proteins. When fused to the heterologous DNA-binding domain of Gal4, the GQ domain can activate transcription in vitro.

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1 The abbreviations used are: SSAP, stage-specific activator protein; 3-AT, 3-aminotriazole; CAT, chloramphenicol acetyltransferase; GOF, gain of function; PCR, polymerase chain reaction; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside.
Mutations That Enhance Transcriptional Activation by SSAP

The Glutamine-rich Activation Domain of SSAP Activates Transcription in Saccharomyces cerevisiae—All glutamine-rich activation domains studied to date fail to activate transcription from integrated reporter genes in the yeast S. cerevisiae (34, 35). Glutamine residues make up 21% (42 of 202) of the GQ domain, solidly supporting its classification as a glutamine-rich domain. Surprisingly, our laboratory has observed that the GQ domain can, in fact, activate transcription in S. cerevisiae to

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after 3 days, transformants were replica-plated to HIS. Formed into L40 yeast and plated on histidine-containing media in conjunction with linearized pBTM116, were transformed by "Experimental Procedures". Mutant plasmids, or mutant fragments of function mutants were isolated. Of the 37 loss of function mutants, 13 were identified in the whole plasmid mutagenesis experiments, whole plasmid or PCR mutagenesis (see under "Materials and Methods" for details). Randomly mutagenized the GQ domain using, in separate culture studies that amino acids 181–290 of SSAP fused to a Gal4 (1–147) DNA-binding domain have no transcriptional activity and that truncation of the C-terminal 30 amino acids of the GQ domain at amino acid 352 severely cripples its ability to transactivate reporter genes (21). Acidic Mutations Enhance the Transcriptional Activity of the GQ Domain—Table I lists the twenty-three gain of function mutants, termed GOF 1–23, and the amino acid substitutions that result from the identified DNA base changes. Unexpectedly, we isolated four mutants (GOFs 5, 20, 22, and 23) encoding truncated forms of the GQ domain as gain of function mutants. GOF 5 and GOF 20 contained an A→T transition that introduced a stop codon at residue Lys-369, whereas GOF 22 and GOF 23 contained a C→T transversion that similarly introduced a stop codon at residue Arg-382. The isolation of these mutations, and of GOF 23 in particular, which contains the deletion as the only mutation in its sequence, argues that the final 14 amino acids of the GQ domain are dispensable for full transcriptional activity. Furthermore, the identification of this deletion in a gain of function screen suggests that residues between amino acids 369 and 382 may negatively modulate its transcriptional activity, possibly through the three basic residues that were isolated in conjunction with other changes; for the description of isolated mutants, refer to Table I.

Mutations That Enhance Transcriptional Activation by SSAP—The numbered sequence at the bottom of each row is the amino acid sequence of the wild type GQ domain. The numbering scheme, from 181 to 382, corresponds to the position of the amino acids within the context of full-length SSAP, a 382-amino acid protein. All mutations causing alterations in the amino acid sequence are identified by indicating above the wild type sequence which amino acid has been substituted. This distribution shows the two "hot spots" for mutation at Lys-369 and Arg-382, two basic residues in the C terminus. No distinction is made in this figure between mutations that were isolated as single events and mutations that were isolated in conjunction with other changes; for the description of isolated mutants, refer to Table I.

Using these methods, 37 loss of function mutants and 23 gain of function mutants were isolated. Of the 37 loss of function mutants, 13 were identified in the whole plasmid mutagenesis screen, and 24 in the PCR mutagenesis screen. All 37 contained +1 or −1 frameshifts, leading to missense reads and premature truncation of the protein. The most distal frameshift iso-lated was at position 310 (see Fig. 2 for GQ amino acid sequence). Thus, amino acids between 310 and 382 contain residues critical for GQ-mediated transcription in S. cerevisiae. This is consistent with our previous findings in mammalian cell culture studies that amino acids 181–290 of SSAP fused to a Gal4 (1–147) DNA-binding domain have no transcriptional activity and that truncation of the C-terminal 30 amino acids of the GQ domain at amino acid 352 severely cripples its ability to transactivate reporter genes (21).

Acidic Mutations Enhance the Transcriptional Activity of the GQ Domain—Table I lists the twenty-three gain of function mutants, termed GOF 1–23, and the amino acid substitutions that result from the identified DNA base changes. Unexpectedly, we isolated four mutants (GOFs 5, 20, 22, and 23) encoding truncated forms of the GQ domain as gain of function mutants. GOF 5 and GOF 20 contained an A→T transition that introduced a stop codon at residue Lys-369, whereas GOF 22 and GOF 23 contained a C→T transversion that similarly introduced a stop codon at residue Arg-382. The isolation of these mutations, and of GOF 23 in particular, which contains the deletion as the only mutation in its sequence, argues that the final 14 amino acids of the GQ domain are dispensable for full transcriptional activity. Furthermore, the identification of this deletion in a gain of function screen suggests that residues between amino acids 369 and 382 may negatively modulate its transcriptional activity, possibly through the three basic residues that were isolated in conjunction with other changes; for the description of isolated mutants, refer to Table I.
Mutations That Enhance Transcriptional Activation by SSAP

Table I

| Mutant | Mutations found |
|--------|----------------|
| GOF 1  | K369E          |
| GOF 2  | K369E          |
| GOF 3  | N364I/K369E    |
| GOF 4  | N297D/S202R/N327D/F344Q/K369E |
| GOF 5  | Q237H/Q238E/K369E/R382Δ |
| GOF 6  | Y279N/K369E    |
| GOF 7  | Y212D/N395S    |
| GOF 8  | N295S/D309N    |
| GOF 9  | Q205P          |
| GOF 10 | N204Y          |
| GOF 11 | G207D          |
| GOF 12 | G320D/N329D    |
| GOF 13 | G193D/T339A    |
| GOF 14 | R235G          |
| GOF 15 | Y362F/G363D    |
| GOF 16 | F377L          |
| GOF 17 | R382L          |
| GOF 18 | R382L          |
| GOF 19 | H373Y/R382L    |
| GOF 20 | Q350L/R382Δ    |
| GOF 21 | G219A/G223R/Q351R/N371S |
| GOF 22 | N295S/K369A    |
| GOF 23 | K369A          |

Mutants that increase the transcriptional activity of the GQ domain in yeast

Plasmids encoding LexA-GQ fusion proteins were isolated from yeast colonies that demonstrated, based upon growth phenotypes and blue-white color assays, increased LexA-GQ driven expression of HIS3 and LacZ reporter genes. The entire GQ domain of these plasmids was sequenced, and the amino acid changes resulting from the alterations in each individual DNA sequence are listed below. The mutants are named GOF, for gain of function, and numbered 1–23.

were performed upon the acidic activation domains of Gal4 and VP16. These results suggest that in addition to its sequence identification as a glutamine-rich activator, SSAP shares mechanistic similarities with acidic activators that are enhanced by the introduction of acidity to its domain. These properties of the GQ domain, in turn, may account for its transcriptional activity in S. cerevisiae.

We quantified the relative effects of several mutants in yeast extracts using liquid β-galactosidase assays. This assay indicates that the mutants confer a 2–11-fold increase in transcriptional activity (Fig. 3). More mutations were identified closer to the C terminus of the protein, and overall, these mutations show more significant effects when assayed in isolation. The K369E mutation and the R382L mutation each conferred a 4-fold increase in activity in the absence of other mutations. The combination of multiple acidic mutations appears to have an additive effect on the fold enhancement (compare, for example, GOF 1 and GOF 5, or GOF 17 and GOF 19). Western blots of yeast extracts show that all the mutant proteins are expressed in roughly equal amounts relative to wild type (data not shown). Furthermore, our screen yielded no false positives; all gain of function mutations isolated in the screen contained, upon sequencing, base changes that altered the predicted amino acid sequence of the encoded protein. This suggests that enhanced transcription of reporter genes is indeed attributable to the altered amino acid(s) and not simply to expression levels. Some of the mutations generate relatively small enhancements (for example, GOF 7 and GOF 15); because of this, we cannot exclude the possibility that some small increases may result from a position effect. Clearly, the generally larger effects and the overall trend of acidic mutations support the hypothesis that acidity increases the activity of the GQ domain.

GQ Domain Gain of Function Mutants Enhance Activated Transcription via a Conserved Mechanism—We next investigated whether the mutations we isolated function via a conserved mechanism by testing the ability of our gain of function mutants to enhance transcription of reporter genes in HepG2 cells. Fragments containing the indicated mutations were cloned into pSG424, allowing expression of mutant GQ domains as Gal4(1–147) fusion proteins. Equal amounts of wild type or mutant DNA were cotransfected with CAT reporter genes containing one (G1E4CAT) or five (G5E4CAT) Gal4 binding sites upstream of the adenovirus E4T promoter (36). We observed that GOF 5, 6, 15, and 17 all increased transcription in HepG2 cells from the G1E4CAT reporter (Fig. 4A). The R382L mutation alone (GOF 17) conferred a 3.7-fold increase over wild type levels, comparable to the effect observed in yeast. GOF 15, a weaker set of mutations in yeast, also retained its ability to enhance transcription. GOF 5 and GOF 6, both of which contain the K369E mutation, increased transcription by 2.7- and 1.5-fold, respectively. Both of these values represent significant drops with respect to the fold activity observed in yeast, but still reflect a gain of function. The difference between them can, as in yeast, be attributed to the presence of other acidic mutations and/or the Arg-382 deletion mutation present in GOF 5 but absent in GOF 6. Western blots show that in mammalian cells, as in yeast, the proteins are expressed at equal levels (Fig. 4B). The ability of these mutations to enhance transcription in mammalian cells as well confirms that the mutants isolated accelerate a conserved step(s) in the pathway toward activated transcription.

When activating transcription from a promoter containing two or more binding sites, the GQ domain exhibits synergistic activation of transcription (37). The mechanisms governing synergistic activation are at least partially distinct from those involving only a single activation domain. We asked whether the same mutants conferred gain of function when transcribing from a promoter containing five binding sites. No mutant tested conferred any significant gain of function upon the G5E4CAT promoter in this assay (Fig. 4C and data not shown). This may simply indicate that under conditions of synergy, the promoter has already achieved maximal activation by SSAP. Alternatively, the synergy of multiple domains may enhance precisely those steps of activation enhanced by the mutations, thereby obscuring their effect in this context.

GQ Domain Gain of Function Mutants Enhance Activated Transcription of the Late H1 Promoter in Sea Urchins—We then turned our attention to the mechanism by which SSAP activates transcription of the late H1 promoter in vivo. Our laboratory has demonstrated that activation of transcription

FIG. 3. Quantitation of transcriptional activity by gain of function mutants in yeast. Quantitation was performed using liquid β-galactosidase assays. For each set of extracts, the transcriptional activity of the wild type domain was set as 1, and all other values are expressed as relative values to the wild type level. All quantitations were performed in triplicate, and the error bars indicate the S.D. of triplicate values to either side of the mean.
Mutations That Enhance Transcriptional Activation by SSAP

In this study, we mutagenized the glutamine-rich transactivation domain (GQ domain) of the sea urchin transcription factor SSAP in search of amino acid motifs critical for, or characteristic of, the ability of this domain to activate transcription. Several observations we have made, in both this and previous studies, argue that the GQ domain fundamentally differs from the classical glutamine-rich domains of Sp1, Oct1, and others previously described. First, the GQ domain activates transcription in S. cerevisiae. Several studies have documented the inability of classical glutamine-rich activators to activate transcription in yeast (34, 35). A few recent studies have led to modifications of this generalization. In Schizosaccharomyces pombe, unlike in S. cerevisiae, glutamine-rich activators function comparably to their acidic and proline-rich counterparts (38). Even in S. cerevisiae, glutamine-rich activators have recently been reported to efficiently activate transcription from vector-based reporter genes (39). In any case, the GQ domain appears to be a unique example of a “glutamine-rich” activator that stimulates transcription of integrated yeast genes.

Second, GQ-mediated transcriptional activation requires from this promoter requires a phosphorylation event on threonine 339, 341, or 343 of the GQ domain of SSAP (46). From a biochemical perspective, the mutations isolated in this screen invite an intriguing comparison to phosphorylation. The K369E mutations, by introduction of a glutamic acid, partially mimic the introduction of a phosphorylated residue at that position. The R382L mutation eliminates a positively charged residue, similarly decreasing the charge of the domain. We therefore tested whether these mutations stimulate transcription mediated by full-length SSAP from its native binding site in sea urchins.

Using appropriately designed PCR primers, we introduced mutations in the C-terminal region of full-length SSAP. The first primer introduced the R382L mutation. The second contained the K369E mutation followed by a stop codon, essentially combining the substitution with the deletion of residues 370–382. As a control, we used a similarly designed primer to amplify the wild type SSAP sequence. This helped confirm that the mRNA could be properly translated without the 3’ untranslated region of the native transcript.

Equal amounts of capped wild type or mutant mRNA were co-injected into fertilized S. purpuratus eggs along with pGC364 (Fig. 5A). This construct responds to microinjected SSAP mRNA in a temporal pattern identical to that of the native late H1 promoter (20). Embryos were harvested at 16 h of development, when transcription of the late H1 gene is at maximal levels, and CAT assays were performed. The results of this experiment are shown in Fig. 5B. In this experiment, wild type SSAP enhanced activity of the late H1 reporter construct by 1.7-fold. This value is slightly below, but within range of, the 2-fold increase we usually observe for microinjection of full-length SSAP mRNA in S. purpuratus eggs. In comparison, the R382L and K369Edel mutations increased transcription by 3.1- and 2.8-fold, respectively; alternatively stated, they conferred gain of function of 82 and 65% relative to wild type levels of transcription. It is important to note that these measurements, both for wild type and mutant SSAP transcripts, were all recorded in the presence of endogenous wild type SSAP activity. Thus, these values probably underestimate the true effect of these mutations in vivo. We conclude that acidic mutations do enhance the activity of the GQ domain in vivo and that these mutations reflect a physiologically meaningful aspect of transcriptional activation by SSAP.

**DISCUSSION**

In this study, we mutagenized the glutamine-rich transactivation domain (GQ domain) of the sea urchin transcription factor SSAP in search of amino acid motifs critical for, or characteristic of, the ability of this domain to activate transcription. Several observations we have made, in both this and previous studies, argue that the GQ domain fundamentally differs from the classical glutamine-rich domains of Sp1, Oct1, and others previously described. First, the GQ domain activates transcription in S. cerevisiae. Several studies have documented the inability of classical glutamine-rich activators to activate transcription in yeast (34, 35). A few recent studies have led to modifications of this generalization. In Schizosaccharomyces pombe, unlike in S. cerevisiae, glutamine-rich activators function comparably to their acidic and proline-rich counterparts (38). Even in S. cerevisiae, glutamine-rich activators have recently been reported to efficiently activate transcription from vector-based reporter genes (39). In any case, the GQ domain appears to be a unique example of a “glutamine-rich” activator that stimulates transcription of integrated yeast genes.

Second, GQ-mediated transcriptional activation requires

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**FIG. 4.** Quantitation of transcriptional activity by gain of function mutants in HepG2 cells. A, activity of the indicated mutants when assayed for transcription from the G1E4CAT reporter. This promoter contains a single binding site for the Gal4(1–147) DNA-binding domain, used in these experiments to target the GQ domain to the promoter. For each set of transfections, the transcriptional activity of the wild type Gal4(1–147)-GQ protein was considered to be 1, and the activities of the mutants were calculated relative to this value. B, activity of the indicated mutants when assayed, following transfection of the indicated quantity of DNA, for transcription from the G5E4CAT reporter, which contains five Gal4 binding sites. For each set of transfections, the transcriptional activity of the wild type protein following transfection of 10 ng of DNA was considered to be 1, and all other values were calculated relative to this value. All transfections were performed in triplicate, and the error bars indicate the S.D. of triplicate values to either side of the mean. C, Western blot of transfected HepG2 extracts probed with anti-bSSAP antibodies to detect Gal4(1–147)-GQ fusion proteins. The lower set of bands, comparing GOF 6 to Gal4-GQ, represents data from a second blot, and the corresponding wild type band from that blot is shown to facilitate the comparison.
Mutations That Enhance Transcriptional Activation by SSAP

Fig. 5. Quantitation of transcriptional activity by gain of function mutants in sea urchins. A, diagram of the sea urchin late H1 promoter construct pGC364 used in this experiment. The promoter contains the full late H1 enhancer, which contains three binding sites for SSAP, upstream of the late H1 basal promoter. The construct also contains three additional SSAP-binding sites downstream of an SV40 poly(A) splice site. In this construct, the promoter directs transcription of the CAT reporter gene. B, activity of the indicated mutants when assayed for transcription from the pGC364 reporter. For each series of injections, all four mRNA samples were injected using the same batch of eggs. For each series, the endogenous activity of the reporter was defined as 1, and all other levels indicate relative activity to that of the reporter. Error bars indicate the S.D. of triplicate values to either side of the mean.

nearly all of its 202 amino acids to function. Our study identified loss of function mutations that truncate the protein after amino acid 310. Previous deletion studies of SSAP, assayed using HeLa cells, have shown that truncations at residue 352 leave a crippled activation domain with activities less than 1% of wild type (21). However, the deleted residues do not independently account for the lost activity; a Gal4 fusion construct with GQ domain residues 291–382 is no more active than that containing residues 181–352 (21). Taken together, these results suggest that SSAP requires a minimal region of 187 amino acids for at least wild type function. In stark contrast, the domains of most other activation domains are modular in structure and require very short segments of the parent protein to retain activation function. Reiterated 18-amino acid segments of the Sp1 domain can effectively reconstitute wild type activity (40). A five-amino acid motif of VP16 can similarly activate transcription when reiterated as few as four times (41). This unusual length requirement of the GQ domain may suggest a higher order structural requirement for activation by SSAP.

Third, the ability of acidic residues to enhance activity suggests more mechanistic similarity with acidic domains than with glutamine-rich domains. Close inspection of the normal SSAP sequence fails to reveal any acidic motifs in the GQ domain; indeed, only 6 out of 202 residues are aspartic or glutamic acid. Yet acidic substitutions throughout the domain manage to enhance its transcriptional activity. The panel of mutations isolated in this screen closely parallels the assortment of acidic mutations isolated in previous yeast screens for mutations to acidic domains (see, e.g. Ref. 9). One possible interpretation of this finding might suggest that these mutations reflect the bias of the yeast system toward acidic activators over glutamine-rich activators and that these mutations represent the artificial consequence of this bias. However, the fact that the wild type GQ domain activates transcription in yeast suggests that the enhancements to transcription conferred by mutations reflect enhancements the natural ability of this domain to mediate transcriptional activation. It is unlikely that these substitutions, in the context of the GQ domain, artificially introduce a new mechanism through which the GQ domain can now activate transcription in addition to its preexisting one(s). Additionally, the ability of several mutations to enhance GQ domain function in mammalian cells and sea urchins supports the argument that this battery of mutations, although initially isolated in a yeast screen, bears physiological relevance for the activity of the GQ domain in its natural context.

Fourth, the ability of the potent GQ domain to synergistically activate transcription to maximal levels when present in multiple copies at the promoter is a property more characteristic of acidic domains. Synergy results from the ability of a given activator to accelerate multiple steps of preinitiation complex assembly and/or processivity of full-length transcripts (42). Both acidic and glutamine-rich domains can synergistically activate transcription when present in multiple copies on a promoter. However, generally speaking, acidic activators are significantly more potent in this context and saturate a given promoter at lower copy number than do glutamine-rich activators. For example, Gal4-VP16 saturates its promoter when bound to five sites, and addition of more Gal4 sites fails to activate transcription any further (43). In our case, the failure of gain of function mutants to enhance transcriptional activity in the context of five binding sites suggests that the promoter is indeed saturated. Experiments by DeFalco (37) show that indeed, Gal4-GQ approaches saturating levels of transcription from a promoter with only two Gal4 binding sites, and the progression toward saturation with increasing numbers of binding sites is nearly identical to that of Gal4-VP16 in the same experimental context.

The isolation of acidic gain of function mutations is particularly interesting in light of the in vivo mechanism by which SSAP is regulated. SSAP is phosphorylated in a stage-specific fashion at the mid-blastula stage of development. This phosphorylation is required for activation of late H1 gene transcription (46). The mutations isolated in this screen mimic the biochemical effect of phosphorylation in that they increase the...
overall negative charge of the activation domain. Most phosphorylation events introduce a site-specific change in a protein, often leading to a structural alteration critical for the function of the phosphoprotein. In the case of SSAP, however, this is not likely to be the case. Analysis of the primary sequence of the GQ domain revealed no predicted secondary structural motifs that would be influenced by phosphorylation. As mentioned earlier, most activation domains are unstructured in solution in the physiological pH range. A single phosphorylation is unlikely to effect significant structural alterations in that context. Introduction of glutamic and aspartic acid residues at positions of mutation in the GQ domain sequence does not alter the structural predictions of computer-based algorithms. Structural studies on other activation domains support this conclusion. Most acidic domains are unstructured in aqueous solution at physiological pH and similarly have no predicted structural motifs. Although many have been induced to adopt an α-helical structure in acidic or hydrophobic environments, phosphorylation seems to play no role in aiding this conformational change. Two phosphorylation events on the acidic activation domain of c-Jun dramatically enhance its transcriptional activity in vivo but do not enable the ability of the peptide to adopt an α-helical conformation in solution (44). Phosphorylation of the kinase-inducible domain of CREB is necessary for its interaction with CBP; however, in the absence of CBP, both phosphorylated and unphosphorylated forms of CREB fail to adopt an α-helical structure (45). Furthermore, with respect to the GQ domain, our original screen isolated acidic mutations through-out the domain. Although we have only tested the more C-terminal residues in sea urchins, we feel it is likely that many of the others would have similar effects. The in vivo phosphorylation of SSAP itself can occur on any one of three residues (46), further suggesting that this phosphorylation is not position-dependent modification. Instead, based on this study, we argue that the in vivo phosphorylation of the GQ domain effects transcriptional activation simply through the introduction of negative charge to the domain. This biochemical property, in turn, then enhances one or more critical protein-protein interactions between SSAP and a general transcription factor, an unknown coactivator, and/or the dimerization of SSAP itself.

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