Characterization of the Activation Domains of AP-2 Family Transcription Factors*

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Despite sequence variation, all AP-2 isotypes are capable of activating transcription, which indicates a functional conservation. We used this property to gain a unique insight into the structure and function of the activation motifs of AP-2 family transcription factors. We have precisely localized the activation motif of human AP-2a to amino acids 52–108. Our experiments indicate that similar sequence of amino acids in all AP-2 isotypes except Drosophila AP-2a harbor their activation motifs. Within this sequence, fewer than 36 residues are critical for transcription activation. Our comparison studies and site-directed mutagenic analyses show that these critical amino acids are strategically placed within this sequence. These residues are interspersed with nonessential and influential residues that vary in composition and length, indicating a structural flexibility. The Drosophila AP-2a has its partly conserved activation motif in an extended region about twice the length of other AP-2 isotypes. Our results reveal essential elements of the amino acid composition of activators in general and shed new light on the mechanism of transcription activation.

Transcription factors tightly regulate gene expression in response to intra- and extracellular stimuli, and they often play a central role in determining cell fate by controlling the fundamental mechanism of gene transcription. Transcription factor AP-2a regulates the genes involved in a spectrum of important biological functions. Some of the AP-2a-activated genes are p21WAF1/CIP1 (1), transforming growth factor-α (2), estrogen receptor (3), keratinocyte-specific genes (4), tyrosine kinase receptor gene c-KIT (5), HIV type-1 (6), HTLV-I (7, 8), type IV collagenase (9), SV40 enhancer region, human metallothionein gene IIa (10), HER-2/neu (11, 12), insulin-like growth factor-binding protein-5 (13), and the dopamine β-hydroxylase gene (14). AP-2a also negatively regulates a number of genes, including MCAM/MUC18 (15); c/EBP-α, during adipogenesis; and c-myc (16). Retinoic acid, a developmental morphogen that is involved in vertebrate limb bud pattern formation, induces AP-2α gene expression transiently in the teratocarcinoma cell lines N-Tera-2 (17, 18) and PA-1 (19). AP-2α mediates transcriptional activation in response to two signal transduction pathways: the phorbol ester/diacylglycerol-inducible protein kinase C pathway and the cAMP-dependent protein kinase A pathway (20, 21).

Aberration of AP-2 activity profoundly affects the cell and the organism. AP-2a null mice have multiple congenital defects at birth, including defective skin and craniofacial abnormalities (22, 23). Mice lacking AP-2β die in early postnatal days from enhanced apoptotic cell death of renal epithelial cells (24). A growing number of studies attribute abnormal AP-2 activity to cancer progression. Their discoveries include the following: a critical role for AP-2α in ras-oncogene-induced transformation of the human teratocarcinoma cells PA-1 (25); constitutive expression of AP-2α in SV40 large T antigen-immortalized human lung fibroblasts unlike in normal cells, indicating a relationship between AP-2α expression and immortalization (26); overexpression of insulin-like growth factor II in rhabdomyosarcoma is caused by AP-2α (27); overexpression of HER-2/neu in breast cancer was induced by AP-2α and AP-2γ (12); regulation of estrogen receptor by AP-2γ in hormone-responsive mammary cancer (3); and AP-2α and AP-2γ regulation of various growth factor signaling pathways that play a role in breast cancer (28); dysregulation of AP-2α causes aberrant expression of c-KIT and MCAM/MUC18 in malignant melanoma (29); and AP-2α correlates with low p21 expression, malignant transformation, and tumor progression in cutaneous malignant melanoma (30).

We made a GAL4-hAP-2α fusion protein in which the activation domain (AD)3 of hAP-2α was linked to a heterologous DNA-binding domain (DBD) of GAL4 and found that it retained the oncogenic property of AP-2 (25). Nontumorigenic PA-1 cells constitutively overexpressing GAL4-hAP-2α induced tumors in nude mice similar to the tumorigenic ras PA-1 cells and hAP-2α-overexpressing PA-1 cells (31). These observations suggested that the AD of hAP-2α mediated tumorigenicity. Our studies indicate that the coactivator PC4, which interacts with the AD of hAP-2α suppresses tumorigenicity in ras-transformed cells (31). Therefore, a detailed analysis of AD of hAP-2α is crucial to understanding its role in tumorigenicity and to identifying additional factors that interact with this region and participate in transformation. Several forms of AP-2 have been isolated from various species, and they constitute a family of transcription factors. AP-2α has been isolated from human (hAP-2α) (32), murine (mAP-2α) (33), chicken (cAP-2α) (34), Drosophila (dAP-2α) (35, 36) and Xenopus (xAP-2α) (37). AP-2β has been isolated from human (hAP-2β) (11), murine

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1 The abbreviations used are: AD, activation domain; DBD, DNA-binding domain; CAT, chloramphenicol acetyltransferase; hAP-2α, human AP-2α; mAP-2, mouse AP-2; xAP-2, Xenopus AP-2; cAP-2, chicken AP-2; dAP-2, Drosophila AP-2; PCR, polymerase chain reaction.
(mAP-2b) (38), and chicken (cAP-2b) (39). AP-2γ was isolated from human (hAP-2γ) (3, 11) and murine (mAP-2γ) (40). All forms of AP-2 show striking amino acid sequence conservation at their C termini in the region of the DBD and, as expected, they bind to same-target sequences. A comparison of the DNA-binding sites of hAP-2α and hAP-2γ was made recently, and their consensus-binding site was determined to be (G/C)(CC-NN)/(A/C/G)/(G/A)G/(G/C/T) (41). The conservation of amino acid sequences occurs relatively less in the N termini, but despite the resulting sequence variation, all forms of AP-2 possess an AD at their N termini. This observation presents a unique opportunity to understand the nature and constitution of the activation motif of AP-2 by comparing the functional role of conserved amino acid sequences. In this report, we have characterized the activation motif of hAP-2α by precisely identifying the amino acids required for its function. The corresponding sequences of other isotypes from various species were also examined to gain insight into the activation property of the AP-2 family of transcription factors.

EXPERIMENTAL PROCEDURES

Cell Culture—NIH 3T3 and COS-1 cells were grown in modified Eagle’s medium with Earl’s salts (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Hazelton Biologics, Lenexa, KS) and antibiotics at 37 °C in 5% CO2, 95% air. 9117 cells, a subline of PA-1 human teratocarcinoma (42), were grown in similar conditions but supplemented with 5% fetal bovine serum.

Transient Transfection and CAT Assays—Transient transfections were performed using SuperFect reagent (Qiagen Inc., Valencia, CA) in NIH 3T3 and COS-1 cells and calcium phosphate precipitate (43) in PA-1 cells grown on 100-mm dishes to introduce DNA. The amount of DNA used in all transfections was equalized by the addition of pBlue-script DNA. 1 μg of β-galactosidase expression vector pCH110 (Amer sham Pharmacia Biotech) was included in each transfection, and the efficiency of transfection was normalized after assaying the sham Pharmacia Biotech) was included in each transfection, and the CAT activity in normalized protein was measured by converting the amino acids required for its function. The corresponding sequences of other isotypes from various species were also examined to gain insight into the activation property of the AP-2 family of transcription factors.

RESULTS

Conservation of Amino Acids in the AP-2 Family and Their Efficiency of Transcription Activation—Isotypes of AP-2 transcription factor AP-2α, AP-2β, and/or AP-2γ have been identified from human, murine, chicken, Drosophila, and Xenopus systems (3, 11, 32–36, 38–40). All forms of AP-2 show strong amino acid sequence conservation near their C termini beginning with the glutamine amino acid. The position of the glutamine residue in hAP-2α is 209. As shown in Fig. 1, the amino acid sequence of hAP-2β is 93% and that of hAP-2γ is 84%, both of which are similar to hAP-2α. Their DBDs are situated in this region, and, as expected, they bind to same target sequences. The sequence is relatively less conserved at their N termini where they contain their ADs. The hAP-2β and hAP-2γ isotypes are 66 and 63% similar to hAP-2α, respectively, in this region. Interestingly, isotype-specific sequence conservation is retained across many species, especially in human, mouse, and chicken. Mouse and chicken AP-2α and AP-2β isotypes are very similar to their human counterparts, mAP-2β is very similar to hAP-2γ, and xAP-2β is very similar to hAP-2α. DAP-2α, however, is significantly less conserved. All are better conserved in the region of their DBDs than their N termini. Irrespective of sequence variation at their N termini, all are able to activate transcription. We used this property to examine the sequences responsible for transcription activation.

We cloned AP-2 isotypes that showed variation in sequence at their N termini in a mammalian expression vector pSG5 under the control of an SV40 promoter to have an identical backbone. An AP-2 reporter construct 3× AP-2hMt-CAT, containing three AP-2-binding sites derived from the human metallothionein gene Ia promoter, was used to measure AP-2 activity in NIH 3T3 cells. Expression plasmids of AP-2 were cotransfected with the reporter construct, and AP-2 activity was determined. As shown in Fig. 2, hAP-2α activated transcription 2-fold above the endogenous level when 1 μg of its expression plasmid DNA was transfected. The dAP-2γ was the strongest activator with about 10-fold induction. In these experiments, xAP-2α activated transcription about 7-fold, cAP-2α 2-fold, mAP-2β 3-fold, cAP-2β 4-fold, hAP-2γ 4-fold, and mAP-2γ 6-fold. We examined their activities using a different AP-2 reporter construct, 3× AP-2SV40-CAT, with AP-2-binding sites derived from an SV40 promoter and found that the pattern of their activation was similar (data not shown). We tested their activity in the human teratocarcinoma cell line PA-1 using 3× AP-2SV40-CAT and observed similar activation of transcription by AP-2 isotypes (Fig. 2). mAP-2β and cAP-2β activation was nearly 2-fold stronger than that observed with 3× AP-2hMt-CAT in NIH 3T3 cells. These results indicate...
that all AP-2 are capable of activating transcription, albeit with different levels of efficiency. Minimal Amino Acid Sequence of hAP-2α Required for Activation—

Our next strategy was to narrow the ADs of AP-2 isotypes to the minimal sequences necessary and study their amino acid conservation. First, we selected the hAP-2α, because some preliminary characterization has been done on the AD of this protein (25, 48) and performed a fine structure analysis to precisely identify the minimal amino acid sequence necessary to activate transcription. Later, we used this information to identify sequences in other AP-2 isotypes to verify their activation and to study how the activation function was conserved. The N terminus of hAP-2α that harbors its activa-

FIG. 1. Sequence conservation in the AP-2 family transcription factors. The molecular structure of hAP-2α is shown on top. hAP-2α has AD near its N terminus and DBD near its C terminus with an integral helix-span-helix dimerization motif (48, 55). The sequences were aligned as indicated using the program LALIGN (56). Different levels of homology were readily identifiable in this alignment in three separate regions in each molecule: the N-terminal amino acids corresponding with 1–208 of hAP-2α, the region of their DBDs, and the C-terminal end (about 40 amino acids). The three regions in each molecule are individually darkly shaded, indicating strong homology to light, which means less conservation of amino acids. The numbers and positions of the amino acids compared are shown below each molecule. The amino acids Asp and Glu; Ile, Leu, and Val; Phe and Tyr; and Lys and Arg are considered similar. I, identity; S, similarity.

FIG. 2. Transcription activation by members of the AP-2 family transcription factors. The cDNAs of AP-2 isotypes were cloned in an expression plasmid pG5. 1 μg of expression plasmid DNA was transiently cotransfected with 4 μg of the AP-2 reporter constructs 3× AP-2hMt-CAT into NIH 3T3 cells or with 2 μg of the AP-2 reporter construct 3× AP-28V40-CAT into 9117 PA-1 cells. CAT activity was determined as described under “Experimental Procedures.” The endogenous AP-2 activities in these cells were considered as one to calculate fold induction. The experiments were repeated at least three times.

The Activation Domains of AP-2 Family Transcription Factors

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| Homology of Human AP-2 Isotypes | Activation | Dimerization |
|---------------------------------|-----------|-------------|
| hAP-2α                          |           |             |
| hAP-2β                          | 1–217     | 218–418     |
| hAP-2γ                          | 1–221     | 222–424     |

Inter-Species Homology

| Homology of hAP-2α | Activation | Dimerization |
|--------------------|------------|--------------|
| mAP-2α             | 1–208 (98.5% I & S) | 411–457 (96.2% I & S) |
| cAP-2α             | 1–208 (97.5% I & S) | 411–457 (96.2% I & S) |
| xAP-2α             | 1–197 (97.8% I & S) | 400–425 (80.8% I & S) |
| dAP-2α             | 1–238 (98.1% I & S) | 441–465 (82.5% I & S) |

Homology to hAP-2β

| Homology to hAP-2β | Activation | Dimerization |
|---------------------|------------|--------------|
| mAP-2β              | 1–216 (100% I) | 419–449 (96.7% I & S) |
| cAP-2β              | 1–216 (97.7% I & S) | 419–449 (93.5% I & S) |

Homology to hAP-2γ

| Homology to hAP-2γ | Activation | Dimerization |
|--------------------|------------|--------------|
| mAP-2γ              | 1–220 (95.5% I & S) | 424–449 (92.5% I & S) |

An earlier study (48) and our preliminary observation indicate that removal of the N-terminal 50 amino acids does not affect the activation function of hAP-2α. To precisely find the N-terminal boundary of the sequence that activates transcription, we prepared DNA fragments that varied by one to a few amino acids. These fragments were linked to the GAL4 DBD, and their activation properties were determined. The amino acids between 51 and 117 and between 52 and 117 showed near full efficiency of activation indicating that the
Activation Domains of AP-2 Family Transcription Factors

The amino acids 51–77 fused to GAL4-DBD do not activate transcription. However, constructs containing the amino acids 6–77 and 20–77 activated about one-eighth activity. The weak activation by the amino acids 6–77 and 20–77 when compared with the relatively no activation by the amino acids 51–77 suggest that the region between amino acids 20 and 50 have a weak intrinsic activation property. This region also positively elevates the activity of hAP-2α when the constructs 20–110 and 51–110 are compared. As summarized in Fig. 4A, the amino acid sequence between the minimal activation motif and the leucines at positions 107 and 108 harbors the central activation motif of hAP-2α, which can significantly activate transcription. There are two regions, one between amino acids 20 and 51 and another between 165 and 226, that positively affect hAP-2α activity. The region between amino acids 122 and 165 has a negative effect.

**Activation Motifs of the AP-2 Family of Transcription Factors**—To gain a detailed understanding of the structure and function of the activation motifs of all AP-2 family transcription factors, we identified the region corresponding to the amino acids between 52 and 108 of hAP-2α in other AP-2 isotypes of various species. We PCR amplified the identified regions, linked them to GAL4 DBD, and tested their ability to activate transcription from a GAL4 target sequence. In such experiments, the amino acid sequences will demonstrate their capability of transcriptional activation, and their sequence variation will serve as a natural source of mutations that can be used for analysis. If two forms of AP-2 have 100% identity in this region, such as hAP-2α and mAP-2α or hAP-2β and mAP-2β, only one of them was selected for examination. A comparison of the amino acid sequences of selected AP-2 isotypes and the efficiency of transcription activation by select regions are shown in Fig. 4B. The selected sequences of AP-2 isotypes except dAP-2α are capable of activating transcription albeit with varied efficiency in both NIH 3T3 and PA-1 cells. Their expression is comparable in COS-1 cells (Fig. 4C). The efficiency of their transcription activation, although not identical, is comparable with the relative activities of corresponding full-length AP-2 isotypes from AP-2 target sequences (Fig. 1). These results demonstrate that the significant core activation motif of all AP-2 isotypes except dAP-2α resides in the regions that correspond with amino acids 52–108 of hAP-2α. It should be noted that the amino acid sequence of dAP-2α that lacks an activation motif in this region is poorly conserved (Figs. 1 and 4B).

We aligned the sequences of AP-2 isotypes that activated transcription significantly with varied efficiency in both NIH 3T3 and PA-1 cells. Their amino acid sequences will demonstrate their capability of transcriptional activation, and their sequence variation will serve as a natural source of mutations that can be used for analysis. If two forms of AP-2 have 100% identity in this region, such as hAP-2α and mAP-2α or hAP-2β and mAP-2β, only one of them was selected for examination. A comparison of the amino acid sequences of selected AP-2 isotypes and the efficiency of transcription activation by select regions are shown in Fig. 4B. The selected sequences of AP-2 isotypes except dAP-2α are capable of activating transcription albeit with varied efficiency in both NIH 3T3 and PA-1 cells. Their expression is comparable in COS-1 cells (Fig. 4C). The efficiency of their transcription activation, although not identical, is comparable with the relative activities of corresponding full-length AP-2 isotypes from AP-2 target sequences (Fig. 1). These results demonstrate that the significant core activation motif of all AP-2 isotypes except dAP-2α resides in the regions that correspond with amino acids 52–108 of hAP-2α. It should be noted that the amino acid sequence of dAP-2α that lacks an activation motif in this region is poorly conserved (Figs. 1 and 4B).

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transcription and examined the amino acids (Fig. 4B). A change of amino acid in any one of the molecules in this alignment would suggest that that particular residue is not critical for transcription activation. Thirty-six amino acids were identical or similar in their sequences that range from 57 to 62 amino acids (Fig. 4B), indicating that the critical residues needed for transcriptional activation are among these 36 residues. These sequences did not agree with any known class of transcriptional activator motifs such as acidic, proline-rich, or glutamine-rich activators nor did conform to any structure that that particular residue not necessary for activation in all forms of AP-2. Amino acids that are similar (e.g. Asp and Glu; Phe and Tyr; and Ile, Leu, and Val) are considered as conserved residues. In this alignment, an amino acid that is different, inserted, or missing when compared with the sequence of hAP-2 is calculated as one variation. The activity of GAL4-hAP-2α 52–108 is arbitrarily set to 100% to calculate the efficiency of transcription activation. The amino acid sequence from 75 to 124 of AP-2α, which contained more than 40 variations, is separately aligned with the conserved sequence. C, expression of GAL4-AP-2 isotype fusion proteins in COS-1 cells. The lane numbers correspond to the isotype numbers in B. The experiments were carried out as described in the legend to Fig. 3B.

**Fig. 4.** The activation motifs of the AP-2 family of transcription factors. A, structure of the activation domain of hAP-2α. The N-terminal 11–226 amino acids are shown with the central activation motif and the regions that positively or negatively affect activation. B, the activation motifs of AP-2 family members and an alignment of their sequences. From all isotypes of AP-2, the region corresponding with amino acids 52 and 108 of hAP-2α were PCR amplified, fused to a heterologous DNA-binding domain (GAL4), and tested for their activation in NIH 3T3 and 9117 PA-1 cells as described in the legend to Fig. 3 and under “Experimental Procedures.” Their sequences are aligned using the Clustal W Multiple Sequence Alignment program (57), and the amino acids that are conserved in all members of AP-2 are highlighted and separately shown below as Sequence Conserved. Variation in any one or more amino acids in this alignment is considered as that particular residue not necessary for activation in all forms of AP-2. Amino acids that are similar (e.g. Asp and Glu; Phe and Tyr; and Ile, Leu, and Val) are considered as conserved residues. In this alignment, an amino acid that is different, inserted, or missing when compared with the sequence of hAP-2α is calculated as one variation. The activity of GAL4-hAP-2α 52–108 is arbitrarily set to 100% to calculate the efficiency of transcription activation. The amino acid sequence from 75 to 124 of AP-2α, which contained more than 40 variations, is separately aligned with the conserved sequence. C, expression of GAL4-AP-2 isotype fusion proteins in COS-1 cells. The lane numbers correspond to the isotype numbers in B. The experiments were carried out as described in the legend to Fig. 3B.
Sequence and examined its activation motif. The GAL4-dAP-2 construct is set to 100% to compare it with the average activity of other mutants of hAP-2α.

| Mutants of AP-2 | Activation % |
|-----------------|--------------|
| hAP-2α          | 100$^a$      |
| hAP-2α D52A    | 12           |
| hAP-2α P56A    | 114          |
| hAP-2α Y57P/F58P | 22        |
| hAP-2α P59A    | 35           |
| hAP-2α L107A/L108A | 2       |
| GAL4-cAP-2β 53–114 | 100$^c$ |
| GAL4-cAP-2β 53–114 N85D | 3   |
| GAL4-cAP-2β 53–114 Q89R | 2   |

$^a$ The activity of wild-type hAP-2α construct is set to 100% to compare it with the average activity of other mutants of hAP-2α.

$^b$ The activity of GAL4-cAP-2β fusion constructs was determined using GAL4 reporter construct G5E1bCAT. The experimental details are described in the legend to Fig. 3.

$^c$ The activity of GAL4-cAP-2β 55–114 is arbitrarily assumed to be 100%.

### DISCUSSION

Several classes of transcription activators have been described: acidic, proline-rich, glutamine-rich, serine-rich, threonine-rich, and isoleucine-rich (see Ref. 51 for a review). The mechanistic role of these residues in transcriptional activation is largely unknown. We sought to understand the structure-function relationship of the AD of AP-2 that is involved in a diversity of important cellular phenomena. The sequence of hAP-2α between amino acids 30 and 120 contains 20 proline and 13 glutamine residues. A synthetic stretch of prolines or glutamines linked to the DBD of GAL4 is capable of activating transcription from the GAL4 target sequence (49). These observations implicitly suggest that the proline and/or the glutamine residues of hAP-2α provide a link to the RNA polymerase II transcription machinery. However, the length of proline-stretch in AP-2 isotypes is significantly shorter, e.g. hAP-2α contains a stretch at position 55 where 6 of 10 amino acids are prolines. In general, transcriptional activators with proline-rich ADs have a length of 9–38 residues, with 80–100% being proline (49). Unlike the glutamine-rich activators, all forms of AP-2 do not contain a glutamine stretch.

Williams and Tjian (48) observed that a hAP-2α construct that lacked the first 50 amino acids retained activation. In their studies amino acids 6–77 of hAP-2α fused to GAL4-DBD activated more than one-fourth of transactivation as compared with amino acids 6–117. The location of a core activation motif of hAP-2α can be discerned, although not conclusively, from these studies to be between amino acids 51 and 77. Our studies clearly show that amino acids 51–77 fused to a GAL4 DBD are incapable of activating transcription, indicating that this sequence of amino acids does not suffice a core activation motif. Our experiments indicate that the 57 amino acids between 52 and 108 of hAP-2α contain the central activation motif that can significantly activate transcription. The boundaries of this sequence are marked by an aspartic acid residue at the N-terminus and by two leucine residues at the C-terminus. Mutations at these positions dramatically affect transcription activation by hAP-2α. Two observations suggest that one leucine suffices.

![Fig. 6. The activation motif of Drosophila AP-2α.](image-url)
at the distal end. The constructs GAL4-hAP-2a/11–107 and GAL4-hAP-2a/51–107 that contain one leucine at their distal ends retain significant ability to activate transcription. The AP-2γ isotypes of human and mouse contain one leucine at their distal ends of their activation motifs. Flanking this activation motif are three regions that influence the activation; two positively and one negatively affect activation. Further experimentation is needed to understand how these regions influence activation.

The sequences of α, β, and γ isotypes of AP-2 from human and other species that correspond with the 57 amino acids of hAP-2α also contain their activation motifs. Their ability to activate transcription is comparable with the relative activities of their full-length molecules on AP-2 target sequences. In the GAL4-AP-2 fusion experiments all the activation motifs are linked to GAL4DBD. This indicates that the variation in their efficiency of activation is not because of varied affinity to the target sequence but rather to the composition of the activation motifs. The conserved residues do not show any resemblance to any known activation motifs of other activators. Comparison of their sequence elucidates the structure and function of these transcription activators. These regions retain their activation motifs despite 3–26 variations in their amino acid composition. All forms of AP-2 contain a DPQ-PPYFPPPPYQ box of sequence at their N termini with minimal variations followed by conservation of residues at specific locations. Altogether, 36 residues are conserved in all forms of AP-2 that activated transcription, indicating that the critical residues needed for transcription activation are among these residues. This region excludes many proline and glutamine residues. An increase in the number of proline residues at the N-terminus of hAP-2α by converting the PPYFPPP sequence to a Pyyyyyyyy sequence negatively affects its activity, indicating that the number of prolines is not the critical determinant of AP-2 transcription activation.

In addition, lowering the number of prolines by altering the proline at position 56 to alanine did not affect activation. Previously, hAP-2α was thought to resemble transcriptional activators CTF/NF-1 and OTF-2 based on the number of prolines they contained (48). Our results and the lack of any further similarity among these activators make this suggestion unlikely.

Other residues that vary in composition and length interrupt the conserved sequence of 36 amino acids. Interestingly, these nonconserved residues influence transcription to various degrees. For example, xAP-2α, which contained three variations from hAP-2α in the nonconserved locations, consistently increased transcription by more than 48%. The strong activators mAP-2γ and hAP-2γ contained 23 and 26 changes, respectively, including insertions and deletions. However, the amino acids needed for activation are not indistinct. The aspartic acid at the N terminus, part of the PPYFPPPPYQ sequence near the N terminus, and at least one hydrophobic residue, leucine, at the distal end of the sequence are essential for transcriptional activation. In addition, two independent alterations in the conserved residues of cAP-2γ, asparagine at position 85 to aspartic acid and glutamine at position 89 to arginine, dramatically affected its activity, emphasizing the need of these amino acids for activation.

The N-terminal sequence of dAP-2 varies significantly from other members of AP-2 family. The similarity is about 33% when compared with hAP-2α. Despite such variation, dAP-2α contains a strong activation motif. The region of dAP-2α corresponding to the 57 amino acids of hAP-2α does not activate transcription. Rather, the activation motif of dAP-2α is borne out by a larger sequence between amino acids 75 and 219. The activation motif of dAP-2α, however, requires some residues that are conserved in other members of the AP-2 family. In summary, the amino acid composition and sequence of the activation motifs of AP-2 accommodate a great degree of variation, and yet they are neither indistinct nor random. One hypothesis is that the AD of AP-2 interacts with coactivators to activate transcription. Many coactivators are involved in polymerase II transcription (52–54). The flexibility of the amino acid sequence of the AP-2 activation motif enables interaction with some common and some different coactivators. The efficiency of activation is determined by the coactivators they interact with and their affinity with them. An implicit suggestion of this hypothesis is that transcriptional activators are merely bridge molecules that provide links to the transcriptional machinery and the coactivators and that the transcriptional activators do not activate transcription on their own. The greater variability of the amino acid sequence of AP-2 activation motifs suggests that an amino acid sequence becomes a transcriptional activator when it is able to interact with a single or a set of coactivators involved in transcription. Hence, the amino acid composition and sequence requirement for an activation motif is less stringent but not indistinct. The activators rich in acidic, proline, or glutamine residues might suggest that they preferentially interact with certain coactivators. The DBD of transcriptional activators binds to a specific DNA target sequence. The coactivators tethered by their ADs enable the assembly of robust transcription machinery near the target gene to modify chromatin structure and augment gene transcription. In this regard, characterization of the tertiary structure of the activation motifs of AP-2 alone will not provide definitive information. Future studies of activator-coactivator interaction and co crystallization of the activator-coactivator complexes is necessary to understand their structural and mechanistic roles in gene transcription. We have shown that the coactivators, e.g. PC4, which interacts with the N-terminal region of AP-2, can be used as tools to suppress cancer (31). Examination of the tumorigenic properties of AP-2 isoforms and their interaction with various coactivators will shed light on the mechanistic steps of tumor progression and open new vistas of cancer suppression.

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