J. Biochem. 126, 130-136 (1999)

[1]AP-2 Enhances Sp1-Dependent Activation of the Growth-Regulated Human ATP/ADP Translocator[1]

Ronggui Li,[2] Zdenek Hodny, Ahmed Zaid, Peter Barath,[3] and B. Dean Nelson

Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, Stockholm, S-106 91 Sweden

Received February 15, 1999; accepted April 21, 1999

The mammalian ATP/ADP translocator isoform-2 (ANT2) gene is growth-activated. Regulation of the gene appears to involve Sp1, as an essential activator, and a suppressor through an Sp1 core element next to the transcription start. We show here that the proximal promoter also binds AP-2 strongly and specifically to two sites, one of which overlaps the Sp1 proximal suppressor site. AP-2 binds with an affinity of 10 to15 fold higher than that of Sp1. AP-2 alone does not alter the ANT2 promoter activity in transfected SL2 cells, but enhances the Sp1-dependent activation of the promoter several fold. Enhancement by AP-2 is observed only when Sp1 is limiting for transcription activation. These data suggest that the cellular AP-2/Sp1 ratio might influence ANT2 expression in developing or differentiating cells.

Key words: ANT2, AP-2, gene expression, Sp1, transcription regulation.

The adenine nucleotide translocator (ANT) is one of the most abundant proteins in the inner membrane of the mitochondrion. It catalyzes the exchange of cytosolic ADP for mitochondrial ATP, thus serving a central function in providing energy to drive extramitochondrial reactions. Three isoforms of ANT have been described for mammalian (*1-4*) and yeast cells (*5, 6*), all of which are encoded by separate genes. The mammalian isoforms are expressed in a tissue-specific manner (*7-11*). In addition, one isoform, ANT2, is expressed in a growth-dependent manner, and was initially cloned as an early immediate gene expressed in growth-activated cells (*12*). The physiological significance of the different ANT isoforms is still not understood, although present evidence suggests that their kinetic parameters differ slightly (*13-15*). In yeast, it has also been shown that one isoform is expressed specifically under anaerobic conditions (*15*).

In an attempt to understand the regulated expression of the human ANT2 gene, we characterized its promoter region (*16*). Activation of the ANT2 promoter was found to rely upon Sp1 sites located in the GC-rich proximal promoter region. We also found that Sp1, when bound to a cognate element adjacent to the transcription start, could suppress transcription initiation (*16*). Thus, human ANT2 expression is both activated and suppressed by Sp1. This unique dual function of Sp1 could play a role in the modulated expression of the ANT2 gene during growth activation. However, a computer search of the proximal promoter region also disclosed two putative AP-2 binding sites in the proximal promoter; a perfect consensus site [GCCN3CGG (*17*)] located between two distal Sp1-activation elements, and an AP-2 like site that overlaps the proximal Sp1 suppressor site (*16*). In view of reports that retinoic acid induces AP-2 (*18-20*) and inhibits ANT2 expression (*12, 21*), we decided to examine the direct effects of AP-2 as a modulator of the ANT2 promoter. Our data show that AP-2 binds strongly to both putative binding elements in the ANT2 promoter, but, alone, has no effect on promoter activity. In contrast, AP-2 enhances Sp1-dependent activation of the ANT2 promoter several fold. The data raise the possibility that the ANT2 promoter can be regulated by the Sp1/AP-2 ratio.

MATERIALS AND METHODS

Clones and Plasmids—CAT reporter constructs containing wild type or Sp1 site-mutated human ANT2 promoter fragments were described previously (*16*). Promoter constructs containing individual AP-2 site mutations [C Box and AP-2 (−200/−179), Fig. 1] were prepared by PCR amplification. PCR primers carrying the mutations were synthesized. Mutated fragments were amplified by the overlap extension PCR method (*22*) using the wild type ANT2 promoter (−235/+8) CAT construct (*16*) as the template. Amplified fragments were cloned into pCAT basic (Promega) for sequence analysis and transfection. Luc reporter constructs were made by substituting the original p21 promoter of plasmid 9.5-Luc (*23*) with ANT2 promoter fragments from CAT reporter vectors.

Expression vectors DRAcAP-2-9(+) (*24*), SP(RSV) AP-2 (*18*), and pPacSp1 (*25*), and reporter plasmid 3xAP-2-RE-tkCAT (*24*) were described previously. pAc-β-Gal

[1] This study was supported by the Swedish Natural Sciences Research Council.

[2] To whom correspondence should be addressed. Tel: +46-8-162462, Fax: +46-8-153679, E-mail: li@mail.biokemi.su.se

[3] Present address: Cancer Research Institute, Slovak Academy of Sciences, Vlarska 7, SK-833 91 Bratislava, Slovak Republic.

Abbreviations: ANT, adenine nucleotide translocator (ATP/ADP translocator); AP-2, activator protein-2; nts, nucleotides; Sp1, stimulating protein-1.

was supplied by Y. Engström, Stockholm University. pCH110 was from Pharmacia Biotech and pGEM3Zf(−) from Promega. The human recombinant Sp1 (E3391) and AP-2 (E3071) proteins used for electrophoretic mobility shift and DNase protection experiments were purchased from Promega. Antibodies against human Sp1 (Sc-059x), Sp3 (Sc-644x), and AP-2 (Sc-184x) were from Santa Cruz Biotechnology, Santa Cruz, CA. Plasmid DNA was purified according to the Promega Protocols and Applications Guide. DNA sequence analysis was carried out with T7 DNA polymerase (Pharmacia) according to the manufacturer's instructions.

Cell Lines and Media—Drosophila SL2 and mbn-2 cells (*26*) were grown in *Drosophila* Schneider's medium supplemented with 10% fetal bovine serum, 2 mM Glutamax I (GIBCO), 50 U penicillin and 50 μg streptomycin/ml at 25°C. For transfection, 5 ml cell suspensions (1 to 2×10^6 cells/ml) were seeded into 60-mm petri dishes (Falcon Plastics). After 20 h growth, the cells were transfected with 10 μg total DNA by the calcium phosphate method (*27*) at 25°C for 48 h. In all transfection experiments the 3XAP-2tkCAT reporter plasmid was included as a positive control as to the function of the AP-2 expression vector. Chloramphenicol acetyl transferase (CAT) and β-galactosidase activities were measured as previously described (*16*). Luciferase activity was measured as recommended by the manufacturer (Promega Guide). The growth and transfection of human HepG2 cells was performed described previously (*16*).

Electrophoretic Mobility Shift and Supershift Assays, and DNase I Footprinting—Nuclear extracts were prepared from NIH3T3, HeLa, or JEG3 cells (*28*), and the protein concentrations were determined with a BIO-RAD protein assay kit (Bradford) using bovine serum albumin as a standard. Electrophoretic mobility shift and supershift assay binding reactions, and electrophoresis were performed as described (*16*). The DNase I protection assay was performed as described by Promega for the core footprinting system.

RESULTS

The minimum promoter of the growth-regulated ANT2 gene (nts −87/+8) contains 3 Sp1 *cis*-elements (A, B, and C boxes, Fig. 1). The A and B boxes function as synergistic activators and the C box is a unique suppressor site (*16*). We previously demonstrated by means of super shift experiments with nuclear extracts of several cell lines that Sp1 is the factor that recognizes the C Box element (*16*). We show here by EMSA that AP-2 also binds to the C Box. For nuclear extracts of JEG3 cells, two band shifts are observed (Fig. 2), whereas a single band shift is found for HeLa and NIH3T3 cell nuclear extracts. Antibodies against Sp1 cause a shift of the lower mobility band for JEG3 nuclear extracts, while antibodies to AP-2 only shift the higher mobility band. Thus, both Sp1 and AP-2 bind to the

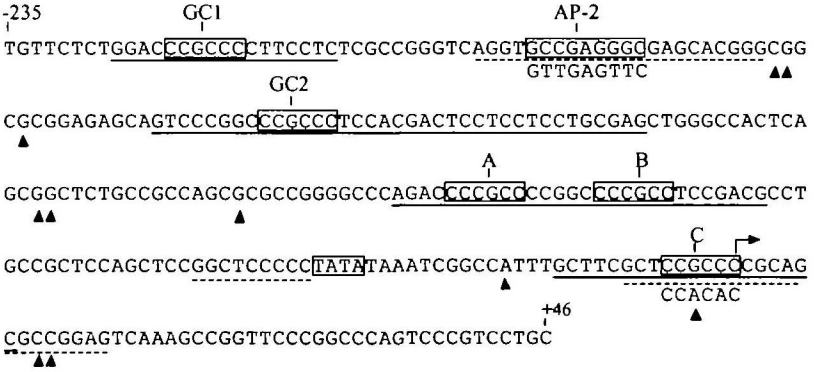

Fig. 1. **Summary of the human ANT2 promoter region.** Consensus Sp1, AP-2, and the TATA elements are boxed. Sequences protected by Sp1 in the DNase I protection assay are underlined with solid lines and those protected by AP-2 are underlined with dashed lines. The mutation introduced into the AP-2 and C box elements are indicated below the elements. The hypersensitive sites induced by AP-2 were denoted with triangles. The transcription start is indicated by an arrow. The A and B boxes are major Sp1 activating elements, and the C box is an Sp1 inhibitory element (*16*).

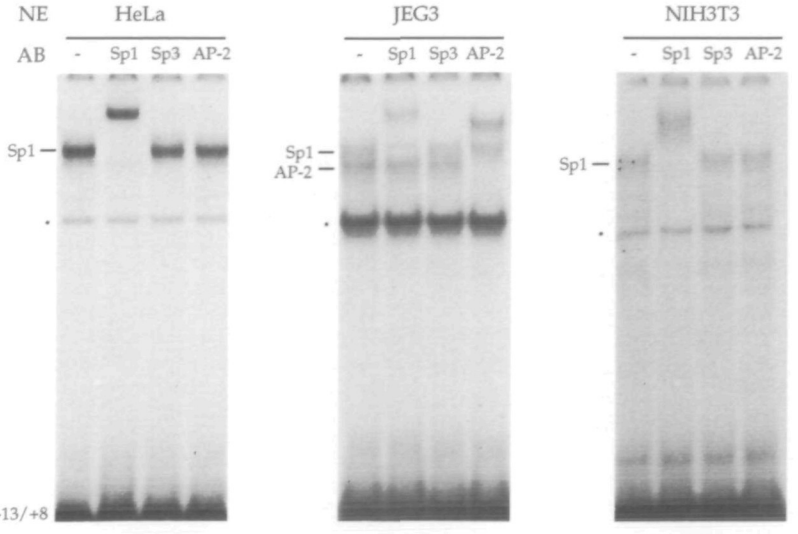

Fig. 2. **Supershift analysis demonstrates Sp1 and AP-2 binding to the C box.** An oligonucleotide covering the C box element (nts −13/+8) was used as the mobility shift probe. The probe was incubated with nuclear extracts of HeLa, JEG3 or NIH3T3 cells, and antibodies against either Sp1, Sp3, or AP-2. Specific Sp1 and AP-2 mobility shift bands are indicated. Nonspecific binding is indicated by dots.

C Box oligonucleotide, but they appear to bind independently of one another. Sp3 binds weakly (*16*), or not at all (Fig. 2).

The relative affinities of Sp1 and AP-2 for the C Box element were examined by means of a competition gel shift assay (Fig. 3). The probe (ANT2 −13/+8) was preincubated with either 0.2 ng of purified human AP-2 (Fig. 3A) or 2.0 ng of purified human Sp1 (Fig. 3B) protein, after which increasing concentrations of either Sp1 or AP-2 protein were titrated. Replacement of AP-2 by Sp1 is seen as an upward shift of the mobility shift band (Fig. 3A), and replacement of Sp1 by AP-2 is seen as a downward shift (Fig. 3B). In both experiments, 50% replacement is observed at an Sp1/AP-2 ratio of about 20–25. We conclude that, after adjustment of the molar ratio of Sp1 and AP-2, AP-2 exhibits about 10–15-fold higher affinity than Sp1 for the C Box element.

DNase I footprinting was performed to confirm the binding of AP-2 and Sp1 to the minimal ANT2 promoter (nts −87 to +46) (Fig. 4A). The purified Sp1 protected the two regions containing the A, B, and C activation/repression boxes. The AB Box region (nts −86 and −59) is more

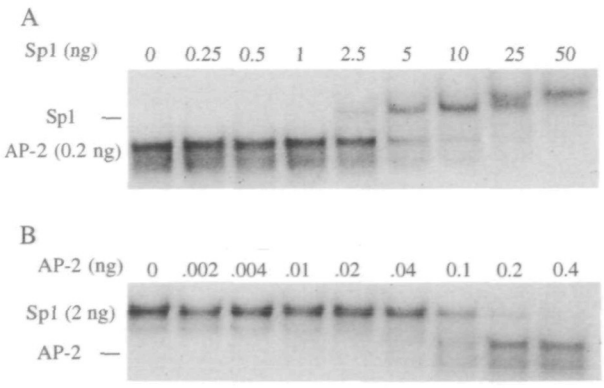

Fig. 3. **AP-2 exhibits higher affinity than Sp1 for the C box element.** Competition binding to the C box oligonucleotide (nts −13/+8) was examined with purified human Sp1 and AP-2 proteins. (A) The probe was incubated with 0.2 ng of AP-2 and titrated with increasing amounts (0 to 50 ng) of Sp1; (B) the probe was incubated with 2 ng Sp1 and titrated with increasing amounts (0 to 0.4 ng) of AP-2. The identities of the shifted bands are indicated.

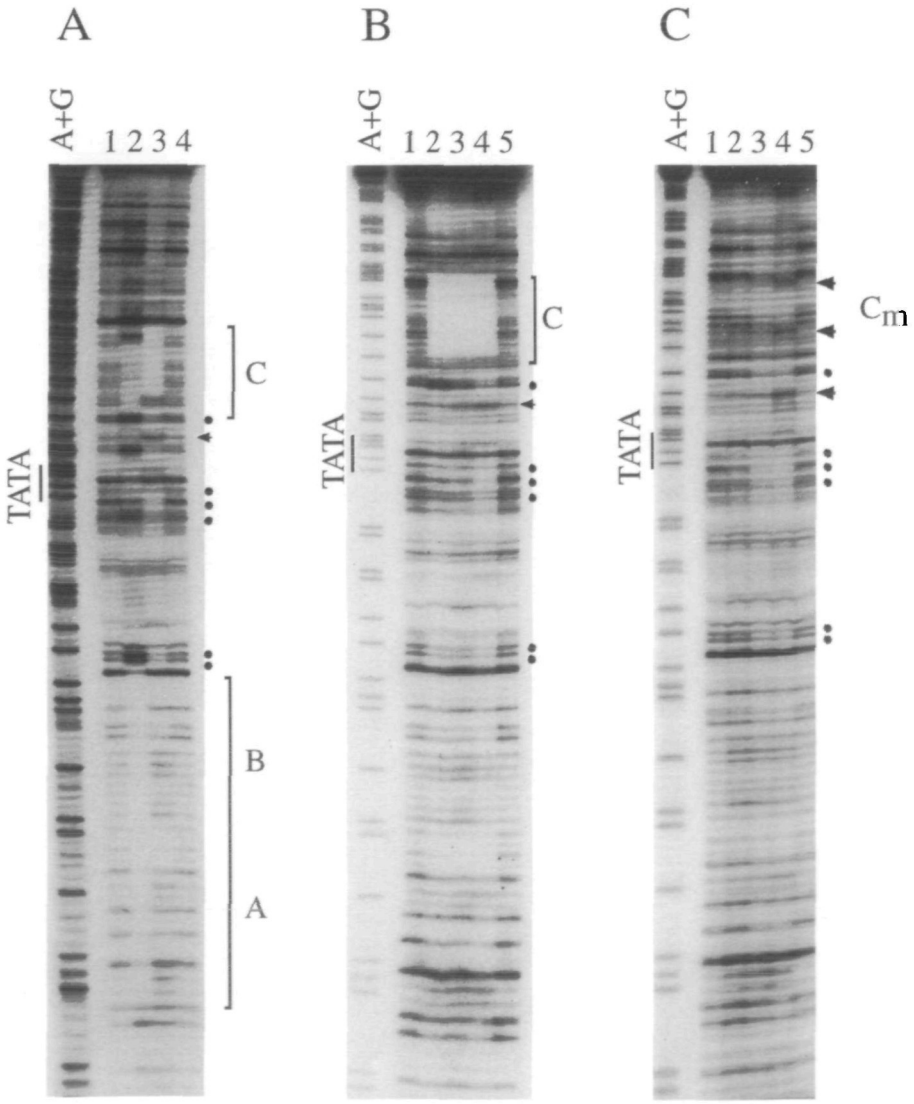

Fig. 4. **AP-2 protects a sequence in the C box that overlaps, but is not identical to, the Sp1 element.** (A) The −87/+46 ANT2 fragment was footprinted in the presence of 40 ng Sp1 (lane 2) or AP-2 (lane 3) (neither Sp1 nor AP-2 in lanes 1 and 4). (B) The −87/+8 fragment, and (C) the −87/+8 fragment with a mutated C box were footprinted in the presence of AP-2 (none in lanes 1 and 5; 10 ng in lane 2; 20 ng in lane 3; and 40 ng in lane 4). The strongly protected regions are indicated by vertical lines on the right. The weakly protected sequences (closed circles) and induced hypersensitive sites (arrows) are indicated on the right. The A+G sequence is on the left.

strongly protected than the C Box region (−14 to +6) (Fig. 4A). AP-2, in contrast, strongly protects a single region (nts −9 to +12) that overlaps the Sp1 C Box binding site (nts −14/+6), but is not identical to it. AP-2 protects the C box region more strongly than Sp1, supporting our conclusion that it binds with higher affinity. AP-2 also weakly protects a region overlapping the 5′-end of the TATA element (Figs. 1 and 4A). Titration with AP-2 reveals that the C box is completely protected by 10 ng protein, while weak protection near the TATA element is discernible only with 40 ng protein (Figs. 4, B and C). Mutation of the core Sp1 element in the C box (CCGCCCC-GCAG to CCACACCGCAG) prevents both AP-2 protection (Fig. 4C) and Sp1 protection (Ref. *16* and data not shown). However, high concentrations of AP-2 protect individual nucleotide and induce hypersensitive sites on the mutated element (Fig. 4C), suggesting weak interactions. In summary, the data show that AP-2 and Sp1 bind to an overlapping site on the C box, utilizing some of the same nucleotides. This finding is consistent with the results of our competition experiments (Fig. 3).

The region between nts −235 and −88 also exerts partial control over transcription of the ANT2 gene (*16*), and contains putative AP-2 and Sp1 sites identified through a computer search. To investigate this region, an extended fragment of the ANT2 promoter (−235/+46) was titrated with Sp1 (Fig. 5A) or AP-2 (Fig. 5B) in a DNase I protection assay. AP-2 strongly protects a region comprising nucleotides −200 to −179 that contains a perfect AP-2 consensus binding element (GCCN3GGC), and weakly protected a site between nts −41 and −35 (Figs. 1 and 5B). Mutation of the consensus element (GCCN3GGC to GTTN3TTC) abolish AP-2 binding (Fig. 5C). Sp1 protects two additional sites, GC1 and GC2, that flank the upstream AP-2 (−200/−179) protected region (Fig. 5A). GC1 (nts −227 to −211) includes a core Sp1 element (CCGCCC), and is the most strongly protected of the two upstream Sp1 sites. GC2 comprises an extended protected region (nucleotides −164 to −128) containing a single core Sp1 element at its 5′-end. Thus, we are able to identify two AP-2 and five Sp1 strongly protected binding sites in the ANT2 proximal promoter.

To determine the functions of the AP-2 elements, cells were transfected with promoters in which one or both AP-2 sites were mutated. We first examined transfected HepG2 cells, which are deficient in AP-2 (*25*). AP-2 alone did not

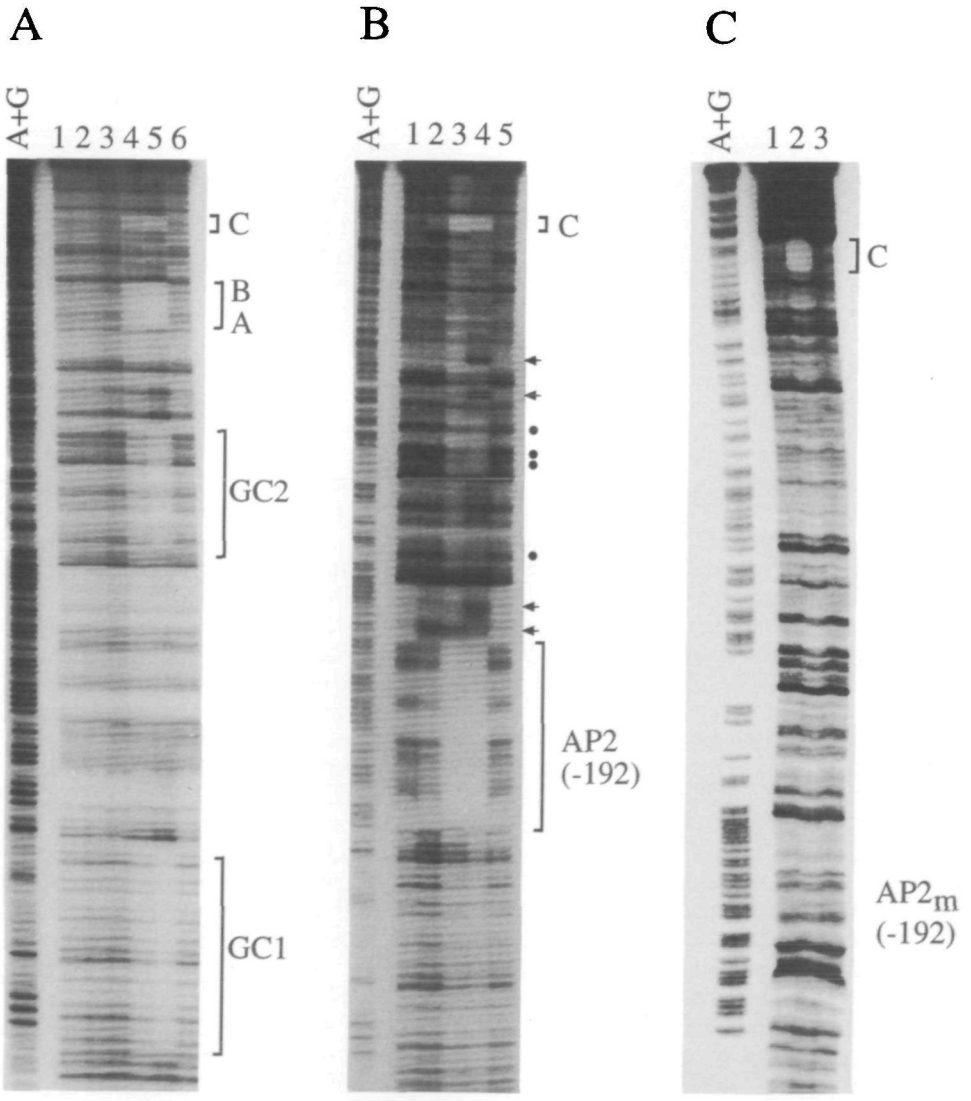

Fig. 5. Identification of AP-2 and Sp1 binding elements in the distal region of the ANT2 proximal promoter. The ANT2 −235/+46 probe was footprinted with increasing amounts of Sp1 in (A) (none in lanes 1 and 6; 5 ng in lane 2; 10 ng in lane 3; 20 ng in lane 4; and 40 ng in lane 5) or AP-2 in (B) (none in lanes 1 and 5; 10 ng in lane 2; 20 ng in lane 3; and 40 ng in lane 4). In (C), the −235/+8 probe bearing a mutated AP-2 element was footprinted with AP-2 (none in lanes 1 and 3; and 40 ng in lane 2).

TABLE I. **AP-2 enhances Sp1-dependent activation of the ANT2 promoter in *Drosophila* cells.**

	Sp1 and AP-2 expression			
	Sp1 (−) AP-2 (−)	Sp1 (−) AP-2 (+)	Sp1 (+) AP-2 (−)	Sp1 (+) AP-2 (+)
Promoter constructs	Fold activation			
Wild type	100	50±2	400±15	1,600±60
C Box Mut	100	60±1	1,200±40	2,500±100
AP-2 (−192) Mut	100	90±4	350±50	2,100±160
3XAP2-tkCAT	100	670±77	n.d.	n.d.

ANT2 (−235/+8) CAT constructs were transfected into *Drosophila* mbn-2 cells. Cells were cotransfected with or without expression vectors for Sp1 (pPacSp1; 0.5 μg) and/or AP-2 [DRAcAP-2(9+); 1 μg]. The 3xAP-2-tkCAT reporter plasmid was included as a positive control for AP-2 expression, and pAc-β-Gal (0.5 μg) was cotrasfected to monitor the transfection efficienty. The data are expressed as fold activation relative to samples with no cotransfected Sp1 and AP-2 expression. The mean±SE are given for at least 3 separate experiments in which each datum was determined in triplicate. C Box Mut, ANT2 (−235/+8) promoter construct bearing the C Box mutation; AP-2 (−192) Mut, ANT2 (−235/+8) promoter construct bearing the distant AP-2 site mutation centered at nt −192; 3XAP2-tkCAT, vector carrying three AP-2 elements in front of the tk promoter driving the CAT reporter gene.

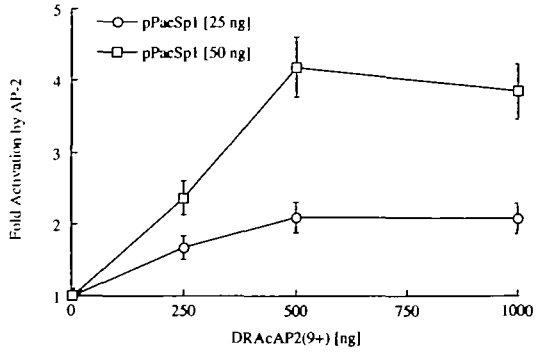

Fig. 6. **AP-2 synergizes with Sp1 in a concentration-dependent manner when both AP-2 sites are mutated.** *Drosophila* SL-2 cells were cotransfected with the ANT2 (−235/+8) CAT promoter construct bearing mutations at both the −192 and C box AP-2 sites, together with increasing amounts of the AP-2 expression vector [DRAc AP-2(9+) in the presence of 25 or 50 ng of the Sp1 expression vector (pPacSp1)]. Transfection efficiency was corrected as to pAc-β-Gal.

significantly alter expression from either the wild type or AP-2 site-mutated promoter (data not shown). Since it is possible that, in these experiments, endogenous Sp1 in HepG2 cells could maintain a high basal promoter activity level and mask weaker effects of the expressed AP-2, we examined the effects of AP-2 in *Drosophila* cells that lack both Sp1 and AP-2 (*18, 25*) (Table I). The results suggest that AP-2, by itself, does not activate the ANT2 promoter in mbn-2 cells (Table I) or SL-2 cells (data not shown).

Since the ANT2 promoter is strongly regulated by Sp1, and since AP-2 sites either overlap or are flanked by Sp1 elements, we examined if AP-2 might influence Sp1 control. As shown in Table I, AP-2 enhances Sp1-dependent transcription from the wild type ANT2 promoter several fold. AP-2 enhancement of Sp1-dependent promoter activity was not abolished by mutating the −200/−179 AP-2 consensus element (Table I). Since this mutation abolishes AP-2 binding (see above), we conclude that the high affinity, consensus element is not responsible for the AP-2 enhancement. This finding raises the possibility that AP-2 enhancement occurs through the C box. However, assessment of the role of the C box is made difficult by the complex interplay between AP-2 and Sp1 at this site; Sp1 and AP-2 compete for the same binding site, and Sp1 suppresses the activity when bound. Therefore, activation at the C box by the combination of Sp1 and AP-2 could have many explanations. In the experiments in Table I, mutation of the C box appeared to lower the extent of activation by AP-2. This, however, is due to the derepression caused by Sp1 release which results in an increase in promoter activity. Under these conditions, factors other than Sp1 limit the promoter activity. This conclusion is supported by the data in Fig. 6, in which it can be seen that AP-2 enhances Sp1-dependent transcription in a concentration dependent manner when lower levels of pPacSp1 (50 ng) are transfected. Figure 6 also shows, surprisingly, that AP-2 enhancement occurs when both stronger AP-2 elements are mutated. However, an approximately 10-fold higher AP-2/Sp1 ratio was required for AP-2 enhancement *via* the mutated C box (Fig. 6) than was required for enhancement *via* the wild type C box (Table I). These data suggest that AP-2, even when weakly associated with the C box, might enhance Sp1-dependent activation from the A and B boxes if Sp1 binding to the C box is prevented. The results also underline the possible importance of the AP-2/Sp1 ratio, and the competition between AP-2 and Sp1 binding to the C box in the regulation of ANT2 expression.

DISCUSSION

We previously showed that primary regulation of the human ANT2 promoter occurs through a unique combination of Sp1 activating/inhibiting elements (*16, 29*). One Sp1 element (C box, see Fig. 1), next to the transcription start, functions as a suppressor element when occupied by Sp1, while four distal Sp1 elements account for activation of the promoter (*16*). Two of the activating elements, the A and B boxes, act in a synergistic manner (*16*). We show here that AP-2 also binds strongly to two sites within the proximal ANT2 promoter, one of which overlaps the Sp1 C box suppressor site. Mutations introduced into the core Sp1 C box element prevent the binding of both Sp1 and AP-2, indicating that they share a common binding site. AP-2 strongly enhances Sp1-mediated activation of the ANT2 promoter in transfected *Drosophila* cells. AP-2 alone, however, appears to have little or no influence on the ANT2 promoter. Interestingly, AP-2 also had little or no effect on the ANT2 promoter when expressed in AP-2 deficient HepG2 cells. One explanation for this lack of effect may be that the level of endogenous Sp1 is already sufficient to assure maximal activation of ANT2. Such a possibility is supported by our studies with the *Drosophila* model system that demonstrated the importance of the relative levels of AP-2 and Sp1 on their synergistic interaction. AP-2 enhances Sp1-dependent transcription only when Sp1 is limiting. These data raise the possibility that, as demonstrated for the K3 keratin gene (*30*), the AP-2/Sp1 ratio may influence ANT2 expression in a tissue- or developmentally-specific manner. This ratio can change dramatically since AP-2 (*20, 31-33*) and Sp1 (*34*) are both expressed in tissue- and developmental stage-specific manners; a difference in the level of Sp1 of 100-fold has

been demonstrated (*34*). Thus, tissues expressing low levels of Sp1 and relatively high levels of AP-2 could come under the control of AP-2.

The precise mechanism by which AP-2 synergizes Sp1 is difficult to predict at the present time due to the complex array of potential interactions between the two. Our data suggest, however, that AP-2 is able to activate from the C box as long Sp1 is not bound, which is consistent with the role of AP-2 as a positive regulator under conditions in which the AP-2/Sp1 ratio is high. This could only be demonstrated, however, for C box mutants which prevented Sp1 binding and reduced AP-2 binding to the C box element. Though difficult to prove at present, we assume that AP-2 would act as an even stronger activator when the C box is intact and the Sp1 level is relatively low. However, it is also possible that the effect of AP-2 is mediated *via* a third DNA site. AP-2 induces a weak protected site in an AT-rich region adjacent to the TATA element that closely resembles the novel high affinity, non-consensus AP-2 binding elements selected from random oligonucleotides by the CAST'ing technique (*35*). One outcome of AP-2 binding to this region could be that it limits access to the TATA element, resulting in a shift of the transcription start. Two transcription start sites have been mapped within the ANT2 proximal promoter using HeLa cell nuclear extracts (Hodny and Nelson, unpublished), one downstream from the TATA box (+1 in this paper) and one upstream, about 30 nts 3′ of the A and B Sp1-activation boxes (*16*). The mechanisms regulating initiation at these sites, or the relevance of these sites to altered transcription efficiency, have not been elucidated.

Regulated expression of the ANT2 gene has been reported in growth activated (*12*) and differentiating (*8, 12, 36*) cells. Growth activation is associated with increased expression of ANT2, whereas the differentiation of myoblasts (*8, 36*) and HL60 cells (*12*) is associated with decreased ANT2 expression, which is in keeping with the concept that ANT2 expression is growth-related. It is noteworthy that retinoic acid has been reported to decrease the levels of ANT2 transcripts in HeLa cells (*21*) and HL60 cells (*12*), and to increase expression of AP-2 in differentiating embryonal carcinoma (EC) cells lines (*18-20*). Although such data suggest a possible causal relationship between retinoic acid and AP-2 in the down regulation of ANT2, we presently have no evidence implicating AP-2 directly in this process. In fact, we have shown that retinoic acid directly increases expression from the ANT2 promoter (unpublished) in cells co-transfected with RXR and TR expression vectors (*37*), a result which is more in line with the activating effect of AP-2 found here with the *Drosophila* system. Thus, although the physiological functions of the high affinity AP-2 sites present in the ANT2 promoter remain to be determined in growth-activated and differentiating cells, it is possible that AP-2/Sp1 interactions could be important for expression of the ANT2 protein.

We wish to thank R. Buettner [DRAcAP-29(+)], Y. Engström (pAc-β-Gal), R. Tjian (Sp(RSV)AP-2; pPacSp1), and M.A. Tainsky (3xAP-2-RE-tkCAT) for generously supplying the indicated plasmids used in this study.

REFERENCES

1. Neckelmann, N., Li, K., Wade, R.P., Shuster, R., and Wallace, D.C. (1987) cDNA sequence of a human skeletal muscle ADP/ATP translocator: lack of a leader peptide, divergence from a fibroblast translocator cDNA, and coevolution with mitochondrial DNA genes. *Proc. Natl. Acad. Sci. USA* **84**, 7580-7584
2. Houldsworth, J. and Attardi, G. (1988) Two distinct genes for ADP/ATP translocase are expressed at the mRNA level in adult human liver. *Proc. Natl. Acad. Sci. USA* **85**, 377-381
3. Cozens, A.L., Runswick, M.J., and Walker, J.E. (1989) DNA sequences of two expressed nuclear genes for human mitochondrial ADP/ATP translocase. *J. Mol. Biol.* **206**, 261-280
4. Ku, D.H., Kagan, J., Chen, S.T., Chang, C.D., Baserga, R., and Wurzel, J. (1990) The human fibroblast adenine nucleotide translocator gene. Molecular cloning and sequence. *J. Biol. Chem.* **265**, 16060-16063
5. Lawson, J.E. and Douglas, M.G. (1988) Separate genes encode functionally equivalent ADP/ATP carrier proteins in *Saccharomyces cerevisiae*. Isolation and analysis of AAC2. *J. Biol. Chem.* **263**, 14812-14818
6. Kolarov, J., Kolarova, N., and Nelson, N. (1990) A third ADP/ATP translocator gene in yeast. *J. Biol. Chem.* **265**, 12711-12716
7. Powell, S.J., Medd, S.M., Runswick, M.J., and Walker, J.E. (1989) Two bovine genes for mitochondrial ADP/ATP translocase expressed differences in various tissues. *Biochemistry* **28**, 866-873
8. Stepien, G., Torroni, A., Chung, A.B., Hodge, J.A., and Wallace, D.C. (1992) Differential expression of adenine nucleotide translocator isoforms in mammalian tissues and during muscle cell differentiation. *J. Biol. Chem.* **267**, 14592-14597
9. Dummler, K., Muller, S., and Seitz, H.J. (1996) Regulation of adenine nucleotide translocase and glycerol 3-phosphate dehydrogenase expression by thyroid hormones in different rat tissues. *Biochem. J.* **317**, 913-918
10. Doerner, A., Pauschinger, M., Badorff, A., Noutsias, M., Giessen, S., Schulze, K., Bilger, J., Rauch, U., and Schultheiss, H.P. (1997) Tissue-specific transcription pattern of the adenine nucleotide translocase isoforms in humans. *FEBS Lett.* **414**, 258-262
11. Graham, B.H., Waymire, K.G., Cottrell, B., Trounce, I.A., MacGregor, G.R., and Wallace, D.C. (1997) A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat. Genet.* **16**, 226-234
12. Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S.T., and Baserga, R. (1987) Molecular cloning of a cDNA for a human ADP/ATP carrier which is growth-regulated. *J. Biol. Chem.* **262**, 4355-4359
13. Klingenberg, M. (1980) The ADP-ATP translocation in mitochondria, a membrane potential controlled transport. *J. Membr. Biol.* **56**, 97-105
14. Drgon, T., Sabova, L., Gavurnikova, G., and Kolarov, J. (1992) Yeast ADP/ATP carrier (AAC) proteins exhibit similar enzymatic properties but their deletion produces different phenotypes. *FEBS Lett.* **304**, 277-280
15. Drgon, T., Sabova, L., Nelson, N., and Kolarov, J. (1991) ADP/ATP translocator is essential only for anaerobic growth of yeast *Saccharomyces cerevisiae*. *FEBS Lett.* **289**, 159-162
16. Li, R., Hodny, Z., Luciakova, K., Barath, P., and Nelson, B.D. (1996) Sp1 activates and inhibits transcription from separate elements in the proximal promoter of the human adenine nucleotide translocase 2 (ANT2) gene. *J. Biol. Chem.* **271**, 18925-18930
17. Williams, T. and Tjian, R. (1991) Analysis of the DNA-binding and activation properties of the human transcription factor AP-2. *Genes Dev.* **5**, 670-682
18. Williams, T., Admon, A., Lüscher, B., and Tjian, R. (1988) Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. *Genes Dev.* **2**, 1557-1569
19. Luscher, B., Mitchell, P.J., Williams, T., and Tjian, R. (1989) Regulation of transcription factor AP-2 by the morphogen retinoic acid and by second messengers. *Genes Dev.* **3**, 1507-1517
20. Philipp, J., Mitchell, P.J., Malipiero, U., and Fontana, A. (1994)

Cell type-specific regulation of expression of transcription factor AP-2 in neuroectodermal cells. *Dev. Biol.* **165**, 602-614
21. Lunardi, J. and Attardi, G. (1991) Differential regulation of expression of the multiple ADP/ATP translocase genes in human cells. *J. Biol. Chem.* **266**, 16534-16540
22. Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77**, 51-59
23. Zeng, Y.-X., Somasundaram, K., and El-Deiry, W.S. (1997) AP2 inhibits cancer cell growth and activates p21WAF1/CIP1 expression. *Nat. Genet.* **15**, 78-82
24. Kannan, P., Buettner, R., Chiao, P.J., Yim, S.O., Sarkiss, M., and Tainsky, M.A. (1994) N-ras oncogenes causes AP-2 transcriptional self-interference, which leads to transformation. *Genes Dev.* **8**, 1258-1269
25. Courey, A.J. and Tjian, R. (1988) Analysis of Sp1 in vivo reveals multiple transcription domains, including a novel glutamine-rich activation motif. *Cell* **55**, 887-898
26. Samakovlis, C., Asling, B., Boman, H.G., Gateff, E., and Hultmark, D. (1992) In vitro induction of cecropin genes-an immune response in a *Drosophila* blood cell line. *Biochem. Biophys. Res. Commun.* **188**, 1169-1175
27. Di Nocera, P.P. and Dawid, I.B. (1983) Transient expression of genes introduced into cultured cells of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **80**, 7095-7098
28. Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**, 1475-1489
29. Zaid, A., Li, R., Luciakova, K., Barath, P., Nery, S., and Nelson, B.D. (1999) Sp1 plays a central role in the expression of diverse genes of the mammalian oxidative phosphorylation system. *J. Bioenerget. Biomembr.* (in press)
30. Chen, T.T., Wu, R.L., Castro-Munozledo, F., and Sun, T.T. (1997) Regulation of K3 keratin gene transcription by Sp1 and AP-2 in differentiating rabbit corneal epithelial cells. *Mol. Cell. Biol.* **17**, 3056-3064
31. Mitchell, P.J., Timmons, P.M., Hebert, J.M., Rigby, P.W.J., and Tjian, R. (1991) Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. *Genes Dev.* **5**, 105-119
32. Moser, M., Imhof, A., Pscherer, A., Bauer, R., Amselgruber, W., Sinowatz, F., Hofstadter, F., Schule, R., and Buettner, R. (1995) Cloning and characterization of a second AP-2 transcription factor: AP-2 beta. *Development* **121**, 2779-2788
33. Meier, P., Koedood, M., Philipp, J., Fontana, A., and Mitchell, P.J. (1995) Alternative mRNAs encode multiple isoforms of transcription factor AP-2 during murine embryogenesis. *Dev. Biol.* **169**, 1-14
34. Saffer, J.D., Jackson, S.P., and Annarella, M.B. (1991) Developmental expression of Sp1 in the mouse. *Mol. Cell. Biol.* **11**, 2189-2199
35. Gee, M.S., Sarkisian, C.J., and el-Deiry, W.S. (1998) Identification of a novel AP-2 consensus DNA binding site. *Biochem. Biophys. Res. Commun.* **243**, 307-316
36. Lunardi, J., Hurko, O., Engel, W.K., and Attardi, G. (1992) The multiple ADP/ATP translocase genes are differentially expressed during human muscle development. *J. Biol. Chem.* **267**, 15267-15270
37. Li, R., Luciakova, K., Zaid, A., Betina, S., Fridell, E., and Nelson, B.D. (1997) Thyroid hormone activates transcription from the promoter regions of some human nuclear-encoded genes of the oxidative phosphorylation system. *Mol. Cell. Endocrinol.* **128**, 69-75