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The MCK enhancer contains a p53 responsive element

(p53/anti-oncogene/trans-activation)

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ABSTRACT **p53 is an antioncogene that is defective or absent in a large number of human tumors. Its function in normal cells is not known. We show that co-transfection of mouse p53 with muscle-specific creatine kinase–chloramphenicol acetyltransferase reporter gene, containing 3.3 kilobase of upstream control sequence for the muscle-specific creatine kinase gene, results in a 10- to 80-fold activation. The p53 responsive element maps to a region distinguished from the known MyoD binding region. Identification of a p53 responsive element should allow a more focused analysis of the effects of p53 in controlling gene activity.**

Alteration or loss of p53 is associated both with cell transformation in culture and several types of human tumors (1–3). Although the p53 protein product can bind to DNA (4) and contains a transcriptional activation domain (5, 6), no p53-responsive DNA element has been identified nor has a specific DNA binding site been determined.

Activated oncogenes are known to inhibit the ability of MyoD to promote myogenesis (for review, see ref. 7). Because three established cell lines (CV1, HepG2, and HeLa) fail to express muscle marker genes when transfected with MyoD (8) and because many cells in culture are known to contain either no p53 or defective p53, we decided to test whether cotransfected p53 would facilitate the activation of myogenic genes by MyoD in these cells. As reported here, this was not the case; however, the regulatory region of one muscle-specific gene, the muscle-specific creatine kinase (MCK) gene, was found to contain a p53 responsive element that will activate a MCK–chloramphenicol acetyltransferase (CAT) reporter gene 40-fold. Activation occurred in the absence of MyoD, and the p53 responsive element in the MCK enhancer mapped to a position distal to the known MyoD binding sites.

RESULTS AND DISCUSSION

Table 1 shows that cotransfection of CV1 cells with a MyoD vector, wild-type murine p53, and the 3300 MCK–CAT reporter [which contains 3300 base pairs (bp) of the MCK (8) upstream control region] results in a 40-fold stimulation of CAT activity as compared to transfections with MyoD plus MCK–CAT or MCK–CAT alone. However, omission of MyoD and transfection with just murine p53 plus MCK–CAT showed that activation of MCK–CAT in CV1 cells did not require MyoD but did require murine p53. MCK–CAT is also activated by p53 in HepG2 cells and CH3/10T½ (10T½) cells. By using various concentrations of MyoD and p53 vectors, we could show that whereas MyoD had an additive effect, it did not have any synergistic effect with p53 in activating MCK–CAT in either CV1 or 10T½ cells. Human wild-type p53 fails to activate the murine MCK–CAT (not shown) suggesting that activation may be species specific; however, levels of protein produced by the expression vectors were not directly assayed in these experiments. By using an MCK-specific antibody, we have not been able to demonstrate activation of the endogenous MCK gene in 10T½ cells transfected with p53; in contrast 10T½ cells transfected with MyoD express endogenous MCK immunoreactivity.

Table 2 shows that a transforming mutant of murine p53 (Ala → Val at position 135) fails to activate MCK–CAT in CV1 cells. A related mutation has lost its capacity for transactivation when fused to the DNA binding domain of *Gal4* (5, 6). Mutant p53 can yield a dominant negative phenotype when coexpressed with wild-type p53 (10–12). In our transcriptional assay system, a 3:1 ratio of mutant to wild-type p53 inhibits transactivation of MCK–CAT by 80% and, therefore, yields a dominant negative phenotype. However, in stable C2 myoblast cell lines, mutant p53 did not inhibit myogenesis as assayed by muscle cell fusion; in transient assays using 10T½ cells, mutant p53 inhibited transactivation of MCK–CAT by MyoD by only 2- or 3-fold and we could not demonstrate that this small amount of inhibition mapped to a specific location within the 3300-bp MCK control region (data not shown).

To obtain preliminary data on the p53 responsive element in the 3300 MCK–CAT reporter, we assayed the ability of p53 to transactivate a vector containing the 110-bp muscle enhancer (normally located at about position −1200 within 3300 MCK–CAT) driving the MCK promoter or the MCK promoter itself (13). Neither of these constructs nor a construct where the polymerized MyoD binding site (4R-TK–CAT) drives herpes thymidine kinase (TK)–CAT (14) were activated by p53 (Table 1). The p53 responsive element, therefore, lies elsewhere on the MCK–CAT vector. By using a series of 5′ deletions of the MCK enhancer, a p53 responsive region maps between positions −3300 and −2800 (Table 3). This same construct, when assayed in transgenic mice, still yields muscle-specific MCK; however, levels of MCK are diminished by a factor of 10–100 (15). Other muscle-specific reporters (3-kilobase desmin–CAT, 3-kilobase cardiac α-actin–CAT, and 7-kilobase MyoD–CAT) were not activated by p53 (data not shown). A p53 responsive element could be associated with these genes but located elsewhere. p53 did not activate or repress reporter constructs driven by either the murine sarcoma virus long terminal repeat or the simian virus 40 enhancer–promoter (not shown).

These experiments show that p53 can act to transactivate a region between positions −3300 and −2800 within the 3300-bp segment of the MCK upstream control region in a variety of tissue culture cell types. Our assays do not suggest a role for either MyoD or the muscle-specific enhancer, though it seems likely that in the proper cellular context, these elements may participate with p53 in controlling expression. Whether p53 directly binds to elements in the

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Abbreviations: CAT, chloramphenicol acetyltransferase; MCK, muscle-specific creatine kinase.

Table 1. Activation of MCK–CAT by p53

Activator(s)	Cell type	CAT activity			
		3300 MCK–CAT	MCK promoter–CAT	110 MCK–CAT	4R-TK–CAT
None (control vector)	CV1	1	1	1	1
	HepG2	1	1	1	1
	10T½	1	1	1	1
MyoD	CV1	0.2	0.5	0.3	–
	HepG2	0.3	0.4	0.2	–
	10T½	65 (10–80)	3.2 (0.8–5)	42 (7–55)	38 (8–40)
p53	CV1	42 (21–74)	0.6 (0.2–0.9)	0.6 (0.2–0.9)	0.4 (0.3–0.8)
	HepG2	32	–	–	–
	10T½	48	–	–	–
	CV1	38	–	–	–
MyoD + p53	HepG2	36	–	–	–
	10T½	44	–	–	–

Cells were cotransfected with the indicated plasmids and CAT activity was determined (8) relative to controls. Control vectors contained the simian virus 40 enhancer (for p53 controls) or the murine sarcoma virus long terminal repeat (for MyoD controls). For CV1 cells, each entry represents the average from six independent experiments (each done in duplicate) and the values in parenthesis give the extremes. For 10T½ and HepG2 cells, each entry is the average of at least two experiments (in duplicate). 3300 MCK–CAT contains 3300 bp of upstream sequence (including a 110-bp muscle enhancer) from the muscle-specific creatine phosphokinase gene; MCK promoter–CAT contains the MCK promoter (positions −80 to +5); 110 MCK–CAT contains the 110-bp muscle-specific enhancer driving the MCK promoter–CAT; 4R-TK–CAT contains four MyoD binding sites driving the herpes thymidine kinase–CAT; the MyoD plasmid is a MyoD cDNA transcribed from the murine sarcoma virus long terminal repeat; murine p53 is a p53 cDNA transcribed from the simian virus 40 enhancer. All p53 plasmids were obtained from A. J. Levine and Robin Quartin (Princeton University); wild-type p53 is p11-4; mutant p53 is p11-4cG (9). For most experiments, reporter plasmid was at 10 μg per dish; activator plasmid was at 10 μg; and the appropriate control plasmid was used to equalize total DNA in each experiment. Both reporter and activator plasmids saturated expression at about 8 μg per dish as revealed by increasing the levels of one and keeping the other in excess.

Table 2. Dominant negative inactivation of P53 by mutant p53

Activator(s)	CAT activity	
	MCK promoter	3300 MCK–CAT
None (vector)	1.0	1.0
p53	0.3	38
p53 mutant	0.4	1.4
p53 + p53 mutant	0.6	4.2

Analysis was in CV1 cells and is described in detail in Table 1. Entries represent the average from three experiments, each done in duplicate. The reporter was at 10 μg per dish; the activator was at 5 μg; and the competitor was at 15 μg. Vector DNA was used to assure each transfection received a total of 30 μg of DNA per dish.

Table 3. p53 responsive element maps to a specific region of the MCK upstream region

Activator	CAT activity					
	−3300	−2800	−2200	−1647	−1256	−776
None (vector)	1	1	1	1	1	1
p53 (exp. 1)	21	1.7	1.3	1.8	1.6	2.1
p53 (exp. 2)	90	5.3	6.5	7.5	9.0	12.3

The 5′ deletions of MCK–CAT (−3000, −2800, etc.) are described in ref. 13. Conditions for assays are described in the legend to Table 1. Each experiment represents the average from duplicate transfections.

MCK upstream region remains to be determined. Because p53 plays a central role in many normal tumors, its activity with respect to the MCK upstream control region should be useful in identifying a specific function.

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