Integrin-associated protein (IAP) is expressed in a variety of tissues, including the nervous system and immune system. To understand how cells control the expression of the IAP gene, we cloned the 5'-proximal region of the human IAP gene and investigated IAP promoter activity by transient transfection. RT-PCR confirmed the expression of IAP transcripts in human neuroblastoma IMR-32 and hepatoma HepG2 cells. Deletion analysis identified a core promoter of the human IAP gene located between nucleotide positions −232 and −12 relative to the translation initiation codon in these two cell lines. Site-directed mutagenesis and gel electrophoretic mobility shift assay identified a α-Pal/NRF-1 binding element within the IAP core promoter. Supershift assays using the α-Pal/NRF-1 antisera confirmed the binding of this transcription factor on the α-Pal/NRF-1 site. Overexpression of the DNA binding domain of α-Pal/NRF-1 in cells enhanced DNA-α-Pal/NRF-1 binding in vitro. Furthermore, overexpression of full-length α-Pal/NRF-1 significantly enhanced IAP promoter activity while overexpression of dominant-negative mutant reduced promoter activity both in the cultured human cell lines and primary mouse cortical cells. These results revealed that α-Pal/NRF-1 is an essential transcription factor in the regulation of human IAP gene expression.

The abbreviations used are: IAP, integrin-associated protein; NRF-1, nuclear respiratory factor 1; CREB, cAMP response element-binding protein; EMSA, electrophoretic mobility shift assay.

Experimental Procedures

Cell Culture—Human neuroblastoma IMR-32 (CCRC 60014) and hepatoma HepG2 (CCRC 60048) cell lines were purchased from Culture Collection and Research Center, Food Industry and Development Institute, Hsinchu, Taiwan. Cells were grown in minimum essential medium Eagle with Earle's salt base (Sigma) supplemented with 10% fetal bovine serum (Hy Clone, Logan, UT) in a humidified atmosphere containing 5% CO₂ at 37 °C.

RNA Isolation and Reverse Transcription (RT)-PCR—Total RNA was isolated from the cultured cells using TRIzol reagent (Invitrogen). RT-PCR was performed as described previously (3). Briefly, total RNA (2 μg) was reverse-transcribed into cDNA in 20 μl of 1× first strand buffer containing 0.5 μg of oligo(dT) as a primer, 500 μM dNTP, and 200 units of Superscript II (Invitrogen). PCR was performed in 20 μl of 1× PCR buffer containing 2 μl of RT products, 1 unit of AmpliTaq DNA polymerase (Roche Applied Science), 500 μM dNTP, 1.5 mM MgCl₂, 0.5 μM [35S]dATP (Amersham Biosciences), and 0.4 μM primer pair. We used the primer pair that can distinguish the alternative splicing forms of IAP mRNA, Hiap14: 5′-CGT AAG GGT CTC ATA GGT G. The PCR parameters were 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s for 30 cycles, followed by a final elongation at 72 °C for 7 min. PCR products were analyzed on a 6% polyacrylamide-urea gel (acrylamide/bisacrylamide 19:1, 8 μl urea in 1× Tris borate-EDTA buffer). The gel was finally dried and analyzed by autoradiography. The image of cDNA bands was scanned by the ScanJet 4C scanner (Hewlett Packard). The optical densities of cDNA bands were quantified with the one-dimensional advanced Universal Software (American Applied Biotechnology, Fullerton, CA).

Plasmids—pGL3-Basic and pRL-TK luciferase reporter vectors (Promega).
sequence motifs of Sp1 and inserted into pGL3-Basic. The fragments were then digested by KpnI and HindIII and promoters regions and a common vector sequence containing the HindIII site. These fragments were then digested by forward primers on the IAP promoter with the KpnI site at the 5'-end and a common reverse primer (GLprimer2) on pGL3-Basic in the backbone of pGL3-232, pGL3-92, were similarly than the insert in pGL3-232, pGL3-191, pGL3-159, and pGL3-92, were similarly similarly similar.

Transfection and Dual-luciferase Assay—IMR-32 and HepG2 cells were plated onto 6- or 10-cm cultured dishes and incubated for 2 days. The cells were washed in phosphate-buffered saline (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM dibasic sodium phosphate, and 2 mM monobasic potassium phosphate) and the lysates were prepared by scraping the cells from plates in the presence of 1× passive lysis buffer (Promega). Luciferase assays were performed by using Dual-Luciferase Assay System (Promega) and a Sirius luminometer (Berthold Detection System, Pforzheim, Germany).

Preparation of Nuclear Extracts—IMR-32 and HepG2 cells were plated onto 6- or 10-cm cultured dishes and incubated for 2 days. The cells were lysed in 1 ml of phosphate-buffered saline. The cells were centrifuged at 2,000 × g for 2 min, and the supernatant was discarded. The cell pellet was incubated in 400 µl of buffer A (10 mM HEPES (pH 7.9), 1.5 mM magnesium chloride, 10 mM potassium chloride, 0.5 mM phenylmethylsulfonfonyl fluoride, 0.5 mM dithiothreitol, 2 µg/ml leupeptin, 10 µg/ml aprotinin, 50 mM sodium fluoride, and 1 mM sodium orthovanadate) on ice for 10 min and then gently shaken for 10 s. The pellet of the crude nuclei was collected by centrifugation at 12,000 × g for 10 s. The pellet was resuspended in 100 µl of buffer C (20 mM HEPES (pH 7.9), 25% glycerol, 420 mM sodium chloride, 1.5 mM magnesium chloride, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonfonyl fluoride, 0.5 mM dithiothreitol, 2 µg/ml leupeptin, 10 µg/ml aprotinin, 50 mM sodium fluoride, and 1 mM sodium orthovanadate) by vortex for 15 s, and then incubated on ice for 20 min. After centrifugation at 12,000 × g for 2 min, the supernatant containing the nuclear proteins was collected, quantified by Bradford Protein Assay Reagent (Pierce), and stored at —70 °C in aliquots.

Expression of Integrin-associated Protein Gene in Human IMR-32 and HepG2 Cells—To use IMR-32 and HepG2 cells to study the IAP gene promoter, we examined firstly the expression of IAP transcripts in these cells by RT-PCR. We used primers that can detect alternative splicing forms of IAP mRNAs. As shown in Fig. 1A, both form 1 and form 2 IAP mRNAs were expressed in these two cell lines at a similar level. The major form of IAP mRNA in IMR-32 was form 1. But form 2 mRNA was expressed 3-fold of that expressed in HepG2 cells (Fig. 1A).

RESULTS

Expression of Inte…
1B). These results confirmed the expression of the human IAP gene in these two cell lines.

**Determination of IAP Promoter Activity in IMR-32 and HepG2 Cells**—To define the boundaries of a minimal IAP promoter region and identify cis elements that regulate the expression of IAP, we generated a series of 5’-IAP promoter deletion constructs and transfected them into IMR-32 and HepG2 cells. All plasmid constructs were defined relative to the translation initiation codon (Fig. 2A). The reporter constructs were cotransfected into IMR-32 and HepG2 cells with an internal control Renilla luciferase vector. The firefly luciferase activity of each reporter was normalized with the internal control to correct transfection efficiency. Results were represented as a fold-increase in activity with respect to that of the pGL3-Basic vector (Fig. 2, B and C) or the relative activity compared with that of the −232 construct (Fig. 4).

In IMR-32 cells, the shortest reporter that still retained the basal promoter activity was the −232 construct, whereas deletion for another 41 bp (the −191 construct), 73 bp (the −159 construct), or 140 bp (the −92 construct) resulted in markedly loss of reporter activity. Addition of 40 bp to the −232 construct generated the −272 construct and stimulated the reporter activity by −25%. In the construct containing additional 184 bp (the −456 construct), the promoter activity was not increased. Interestingly, the reporter activity decreased to about the basal proximal promoter activity in the construct containing additional 274 bp (the −730 construct) and in the longest construct (the −1554 construct) (Fig. 2B). A similar pattern of promoter activity was observed when the reporter constructs were transfected into HepG2 cells, but with three exceptions (Fig. 2C). First, overall promoter strength relative to pGL3-Basic was much lower in HepG2 cells, about one-sixth to one-fourth compared with IMR-32 cells. Second, the constructs of −272 and −456 produced maximal activity in IMR-32 cells; in HepG2 cells, however, the construct of −730 produced maximal activity. Third, there were negative regulators located between −457 and −730 in IMR-32 cells; in HepG2 cells, however, there were negative regulators located between −730 and −1554. Results suggested a core promoter of the human IAP gene located between −232 and −12 upstream of the translation initiation codon in both IMR-32 and HepG2 cells.

The sequence from −272 to ATG was searched for homology to previously described regulatory elements in several databases. There are many putative binding sites for transcription factor in this region (Fig. 3), including activating enhancer binding protein 2 (AP-2), Myc-associated zinc finger protein (Maz), cyclic-AMP responsive element-binding protein (CREB), transcription factor Sp1, E2 promoter binding factor (E2F), and α-Pal/nuclear respiratory factor 1 (α-Pal/NRF-1).

**Identification of cis-Elements in the Core Promoter of the IAP Gene**—To determine more precisely the core promoter of the IAP gene, we generated several shorter or point mutation constructs. When 14 and 23 bp were deleted from the 5’-end of the −232 construct to generate the constructs of −218 and −209, respectively, the promoter activity of these two constructs was identical to that of the −232 construct in IMR-32 cells. This indicated that the sequence between −232 and −209 played no significant role in IAP gene expression under the current experimental conditions. When 34 bp were deleted from the 5’-end of the −232 construct to generate the −198 construct; however, the promoter activity was markedly reduced by 90% in IMR-32 cells, indicating that the sequence between −209 and −198 might be required for the IAP promoter activity (Fig. 4). As shown in Fig. 3, the region from −232 to −198 consists of a GC-rich sequence that includes the putative Sp1 and α-Pal/NRF-1 sites. Point mutations were introduced into these sites in IMR-32 cells to determine whether these sites were necessary for IAP promoter activity. When four bases of the
Values are presented as the mean ± S.E. obtained from three independent experiments, each conducted with triplicate cultures. *, p < 0.05; **, p < 0.01; ‡, p < 0.001; n.s., not significant.

A. Constructs

| Restriction Sites | Fold Increase in Promoter Activity (versus pGL3-Basic) |
|------------------|--------------------------------------------------|
| Sall             | Luc     | 1     | 1     | 1     | 1     |
| KpnI             | Luc     | 1     | 1     | 1     | 1     |
| Sacl             | Luc     | 1     | 1     | 1     | 1     |
| Xmal             | Luc     | 1     | 1     | 1     | 1     |
| ATG              | Luc     | 1     | 1     | 1     | 1     |

B. IMR-32

| Construct | Fold Increase in Promoter Activity (versus pGL3-Basic) |
|-----------|--------------------------------------------------|
| -232m1    | Luc     | 1     | 1     | 1     | 1     |
| -232m2    | Luc     | 1     | 1     | 1     | 1     |
| -232m3    | Luc     | 1     | 1     | 1     | 1     |

C. HepG2

| Construct | Fold Increase in Promoter Activity (versus pGL3-Basic) |
|-----------|--------------------------------------------------|
| -232m1    | Luc     | 1     | 1     | 1     | 1     |
| -232m2    | Luc     | 1     | 1     | 1     | 1     |
| -232m3    | Luc     | 1     | 1     | 1     | 1     |

**Fig. 2. Identification of the core promoter region of the human IAP gene in IMR-32 and HepG2 cells.** A. promoter constructs. The restriction map of the 5'-flanking region of the human IAP gene is shown at the top. Positions are indicated relative to the translation initiation codon. The promoter constructs are named by the left boundary and have a common 3' end at −12. B and C, luciferase activity of the promoter constructs in IMR-32 and HepG2 cells. 250 ng of independent constructs and pRL-TK plasmids were cotransfected into cells by the calcium phosphate precipitation method. The firefly luciferase activity after 48-h incubation was normalized to Renilla luciferase activity as a transfection control. These values were then normalized to those of pGL3-Basic vector without any insert. Note the different scales of the x-axes in B and C. Values are presented as the mean ± S.E. obtained from three independent experiments, each conducted with triplicate cultures. *, p < 0.05; **, p < 0.01; ‡, p < 0.001; n.s., not significant.

**Fig. 3. The sequence of 5'-flanking region of the human IAP gene.** The sequence spanning from −272 to the translation initiation codon ATG is shown. This region contains putative binding sites for transcription factors such as AP-2, Max, CREB, Sp1, E2F, and α-Pal/NRF-1. However, two Sp1 (TGCGGC) and one E2F site share partial sequences with the α-Pal/NRF-1 site (23). The positions of these sites are indicated by underlines. The plasmids for the luciferase assay were constructed by inserting fragments spanning from nucleotides (indicated by arrows and numbers) to nt −12, relative to ATG, into the firefly luciferase expression vector (pGL3-Basic).

**Fig. 4. Identification of functional cis elements in the IAP core promoter.** Shorter promoter fragments were made by PCR (the −218, −209, and −198 constructs). Mutant constructs were made by site-directed mutagenesis (the −232m1, m2, and m3 constructs). The putative Sp1 and α-Pal/NRF-1 sites are indicated. Independent constructs and pRL-TK plasmids were cotransfected into IMR-32 cells using the same protocol as shown in Fig. 2. The promoter activity was expressed with respect to the −229 construct. *, p < 0.05; **, p < 0.001; unpaired Student’s t test.

Sp1 site was substituted with four T residues to generate the −232m1 construct. No significant effect on the IAP promoter activity was observed (Fig. 4), indicating that Sp1 site in this region was not required for the IAP promoter activity under this condition. The putative α-Pal/NRF-1 site in the IAP promoter is a 12-base tandem-repeat sequence, TGGCGCT-GCGCG. When two bases in each of the repeat sequence were replaced by two T residues to generate the −232m2 and −232m3 constructs respectively, an 80% and a 90% drop in promoter activity was observed (Fig. 4). These results suggested that the consensus α-Pal/NRF-1 sequence, but not the consensus Sp1 sequence, is a functional regulatory element in the IAP promoter in IMR-32 cells.

α-Pal/NRF-1 Is a Transcription Factor Regulating the IAP Promoter Activity—To demonstrate that the consensus α-Pal/NRF-1 site was functional, i.e. that there were endogenous nuclear proteins binding to this region, we performed the EMSA experiment. Nuclear extracts from IMR-32 cells were combined with 32P-fill-in-labeled double-stranded oligonucleotides in vitro. A major band of DNA-protein complex was found in all lanes when the nuclear extracts were incubated with the wild-type IAP α-Pal/NRF-1 probes (Fig. 5a, lane 3 and lanes 5–12), but not with the mutant IAP α-Pal/NRF-1 probes (Fig. 5a, lane 4). No band was found when nuclear extracts were not added into the probes (Fig. 5a, lanes 1 and 2). Competition analysis using a 10- or 60-fold molar excess of unlabeled probes was used to characterize the factor, which specifically binds to this sequence. As expected, the addition of a 10- or 60-fold molar excess of published wild-type consensus α-Pal/NRF-1 element reduced the intensity of these complexes (Fig. 5a, lanes 5 and 6), whereas the addition of the mutant consensus α-Pal/NRF-1 sequence did not (Fig. 5a, lanes 7 and 8). Results suggested that α-Pal/NRF-1 proteins might bind to the IAP α-Pal/NRF-1 element. However, the α-Pal/NRF-1 site is GC-rich and might therefore interact with factors other than α-Pal/NRF-1, such as Sp1 and E2F. We therefore used the unlabeled consensus Sp1 and E2F sequence for the competition experiment. The intensity of the migrating bands was not significantly reduced (Fig. 5a, lanes 9–12). Supershift assays using the anti-α-Pal/NRF-1 antiserum were used to further confirm the binding of the α-Pal/NRF-1 on its DNA element. The migrating bands were weakened when increasing amounts of α-Pal/NRF-1 antiserum were added and supershifted bands appeared (Fig. 5b, lanes 3–5). However, the Sp1 or E2F antibody did not generate any supershifted band (Fig. 5b, lanes 6 and 7), neither did the normal goat serum (Fig. 5b, lane 8). The EMSA experiments also revealed that the DNA binding activity of α-Pal/NRF-1 in IMR-32 cells (Fig. 5c, lanes 1–3) was much higher than that in HepG2 cells (Fig. 5c, lanes 4–6). The oligonucleotide probes and competitors used in the EMSA ex-
Experiments were shown in Fig. 5D. These results strongly suggested that α-Pal/NRF-1 but not Sp1 or E2F binds to the IAP α-Pal/NRF-1 site.

To further confirm that α-Pal/NRF-1 is the major transcription factor that binds to the IAP α-Pal/NRF-1 element, plasmids encoding the full-length or dominant-negative mutant of α-Pal/NRF-1 with or without a Myc tag were transiently transfected into HepG2 cells. The addition of a Myc tag in the C-terminal of α-Pal/NRF-1 is useful for the supershift assay in the EMSA experiments. In HepG2 cells, overexpression of the full-length α-Pal/NRF-1 (Fig. 6A, lanes 4–6) and Myc-tagged α-Pal/NRF-1 (Fig. 6B, lanes 3–5) enhanced the binding of DNA-protein complex in a dose-dependent manner as compared with the mock controls (Fig. 6A, lane 3 and 6B, lane 2). Overexpression of the dominant-negative mutant of α-Pal/NRF-1, which contains only the N-terminal DNA binding domain, did not affect endogenous DNA-protein binding in HepG2 cells but generated an additional band of DNA-protein complex with a smaller molecular weight. The DNA binding activity was strongly enhanced by the overexpression of the dominant-negative mutant (Fig. 6, A and B, lanes 7–9). This discrepancy may be related to the higher transfection efficiency of the plasmid...
Overexpression of the full-length and dominant-negative α-Pal/NRF-1 on the DNA binding activity. Plasmids containing full-length or dominant-negative α-Pal/NRF-1 cDNA sequences were transfected into HepG2 cells by the calcium phosphate method. Empty vector pcDNA3.1 was used as the mock control (Vec). B, supershift analysis of the Myc-tagged fusion proteins. Plasmids containing the Myc-tagged full-length α-Pal/NRF-1 or Myc-tagged dominant-negative mutant were transfected. Monoclonal anti-Myc antibody was used. Arrows indicate the shifted or supershifted bands.

that contained the dominant-negative mutant. In supershift assays, the band of DNA-protein complex containing the α-Pal/NRF-1-myc fusion protein was supershifted when monoclonal anti-Myc antibody was used (Fig. 6, lane 6). The band of DNA-protein complex containing the dominant-negative mutant of α-Pal/NRF-1 fused with the Myc protein fragment was also completely supershifted by the monoclonal anti-Myc antibody (Fig. 6, lane 10). These data strongly suggested that α-Pal/NRF-1 binds to this region (−204 to −193) of the human IAP gene promoter in vitro.

If the α-Pal/NRF-1 protein binds to the α-Pal/NRF-1 element in the IAP promoter in vitro, it should functionally regulate the promoter activity of the IAP gene in vivo. To test this possibility, the full-length or dominant-negative α-Pal/NRF-1 constructs were cotransfected with the reporter construct −232 into IMR-32 and HepG2 cells, and the luciferase activity was measured. As shown in Fig. 7A, overexpression of dominant-negative α-Pal/NRF-1 significantly reduced the IAP promoter activity in a dose-dependent manner in both cell lines, as compared with the mock controls. At the highest dose (50 ng) the promoter activity of the reporter construct was reduced markedly up to 50 and 92% in IMR-32 and HepG2 cells, respectively. In contrast, overexpression of full-length α-Pal/NRF-1 significantly increased the IAP promoter activity in a dose-dependent manner (Fig. 7B). At highest dose (2 μg) the promoter activity of the reporter construct increased up to 3.9- and 5.3-fold at the highest dose (2 μg) in IMR-32 and HepG2 cells, respectively. In Fig. 7B, the relative activity was represented as the percentage of firefly luciferase activity of mock control but not normalized with the Renilla luciferase activity, because the full-length α-Pal/NRF-1 could enhance the activity of the Renilla luciferase by unknown effects. These results confirm that α-Pal/NRF-1 interacts with IAP promoter and regulates its downstream gene expression in vivo.

Regulation of the IAP Promoter Activity by α-Pal/NRF-1 in Primary Cells—To investigate if α-Pal/NRF-1 regulates IAP gene promoter under physiological conditions, we transfected the truncated or mutant human IAP promoter constructs into the mouse primary cortical cells. Results are shown in Fig. 8. Similar to the results observed in IMR-32 and HepG2 cells (Fig. 2), the −232 construct retained the basal promoter activity, but the activity of shorter constructs, −198 and −195 constructs, was reduced 76 and 62%, respectively. The promoter activity of point-mutation constructs, −232m2 and m3, was significantly decreased about 40%, but not the −232m1 construct (Fig. 8A). Overexpression of dominant-negative α-Pal/NRF-1 in the primary cortical cells decreased the IAP gene promoter activity in a dose-dependent manner (Fig. 8B). Overexpression the wild-type α-Pal/NRF-1 in the primary cortical cells significantly enhanced the IAP gene promoter activity (Fig. 8C). These results indicate that the function of α-Pal/NRF-1 in primary cells is similar to that in cell lines.

DISCUSSION

We have identified in the core promoter of the human IAP gene a cis-acting element that contributes to the control of IAP gene expression. This element is a 12-base pair direct-repeat sequence, TGCAGTGCGCG, located from −204 to −193 nu-
cleotides upstream of the IAP translation initiation codon, which is a consensus sequence for the transcription factor α-Pal/NRF-1. α-Pal/NRF-1 belongs to a new class of transcription factors that contain a unique putative basic leucine zipper (bZip) DNA binding domain (24). The consensus sequence recognized by α-Pal/NRF-1 is (T/C)GGC(G/A)NCGGCCCA (20, 21, 24). The reverse complement sequence of the α-Pal/NRF-1 site in the human IAP promoter is CGCGCACCGGCCA, conforming well to the consensus sequence. This DNA element is critical to the expression of the IAP gene not only in the human neuroblastoma and hepatoma cell lines but also in the mouse primary cortical cells.

α-Pal was first discovered as a key transcription factor in the eukaryotic initiation factor 2α gene, which is a target of the post-translation mechanisms when eukaryotic cells respond to growth, metabolic, and other signals (24, 25). It was designated because it binds to a palindromic sequence TGCGCATGCGCA (25). NRF-1 was discovered independently as a nuclear transcription factor that is important for the regulation of mitochondrial genes responsible for modulation of energy transduction (26). Later studies found that the α-Pal/NRF-1 recognition sequence can be identified in many other genes involved in energy transduction, translation/protein turnover, DNA synthesis/repair, and cellular proliferation (24, 27). The α-Pal/NRF-1 shows strong homology with two invertebrate genes, sea urchin P3A2 and Drosophila erect wing gene (eug). Sequence comparison revealed that the half N-terminal region has been evolutionarily conserved. This region harbors the DNA binding, dimerization, and nuclear localization signal domains. The C-terminal halves comprise the bipartite hydrophobic activation domain and have only conserved short patches of homology between the vertebrate and invertebrate members of the family (20, 24). These two genes have been implicated in embryonic or larval development (28, 29). On the other hand, α-Pal/NRF-1 shows 91% identity to its homologue in zebrafish, not really finished (nrf) (30). Both eug and nrf have
been associated with the development of the central nervous system (29, 30).

In the nervous system, we originally found that the IAP/CD47 mRNA level is up-regulated in the rat hippocampus during the processes of memory formation (3). As a critical positive regulator of IAP/CD47 gene, the binding activity of α-Pal/NRF-1 may be increased after learning. Experiments are underway to determine the level and DNA binding activity of α-Pal/NRF-1 in the rat brain during the processes of memory formation. Several important neuronal genes, such as gene encoding GluR2, FMR-1, or synapsin I, also has the α-Pal/NRF-1 element in their promoter region (20, 31, 32). GluR2 is one of the subunits of the AMPA type of glutamate receptors that mediate a large fraction of the postsynaptic response at most fast excitatory synapses in the brain. FMR-1 is the gene that is involved in the fragile X mental retardation syndrome. Synapsin I is one of the most abundant proteins in the presynaptic terminal and is involved in memory function (33, 34). The involvement of α-Pal/NRF-1 in memory functions remains to be determined.

Similar to the ubiquitous expression of IAP across tissues, α-Pal/NRF-1 is also expressed in a variety of tissues (35). One will wonder how IAP would be regulated from such a ubiquitously expressed transcription factor. Our data suggested that the DNA binding activity instead of the expression level of α-Pal/NRF-1 is the determinant of the expression of the IAP gene. In IMR-32 cells, the total IAP mRNA level is about 3-fold of that in HepG2 cells (Fig. 1B). The DNA binding activity of α-Pal/NRF-1 in IMR-32 cells is also about several folds of that in HepG2 cells (Fig. 5C). The higher IAP mRNA level in the neuroblastoma cells is correlated with the higher DNA binding activity of α-Pal/NRF-1 in these cells. However, mutation or truncation of the α-Pal/NRF-1 binding site not only reduced the promoter activity of IAP gene in neuroblastoma cells but also in hepatoma cells. This suggests that α-Pal/NRF-1 is not a transcription factor that specifically regulates the expression of IAP mRNA in neuronal cells. The question will then be what is the function of α-Pal/NRF-1 in neurons? We recently found that overexpression of α-Pal/NRF-1 in the neuroblastoma cells significantly enhances neurite outgrowth. It seems that the regulation of IAP gene by α-Pal/NRF-1 plays a role in neuronal differentiation.

The other question is that if the preferential expression of form 4 IAP in IMR-32 cells or the nervous systems is correlated with the activity of α-Pal/NRF-1. The α-Pal/NRF-1 recognition site is located in the core promoter of the IAP gene. Our results suggested that α-Pal/NRF-1 is responsible for not only the expression of form 4 IAP in IMR-32 cells, but also the expression of form 1 and form 2 IAP in HepG2 cells. Form 4 as well as form 1 and form 2 IAP mRNA are alternative splicing forms of the IAP gene. Other activators or repressors for alternative splicing will be the determinants for the preferential expression of form 4 IAP in the nervous system.

We compared the core promoter sequence of the human IAP gene with the core promoters of mouse and rat IAP genes and found that the sequence of the α-Pal/NRF-1 site is identical in all three species (Fig. 9). This suggested that this site is evolutionarily important for the control of IAP gene expression. Upstream of the α-Pal/NRF-1 site, there are several other conserved regions in these three species. The first is the Sp1 site next to the α-Pal/NRF-1 site. The three sequences here are also identical. The core promoter sequence of the human IAP gene is TATA-less. According to previous reports, Sp1 plays an important role in TATA-less promoters (23, 36). Although mutation or truncation of this Sp1 site did not affect the activity of human IAP core promoter (Figs. 4 and 8), we did find in EMSA experiments that Sp1 factor binds to this site (data not shown). This implied that Sp1 has the potential to regulate the IAP gene under some conditions or in other cells. The next is the region from −236 to −225 of the human IAP promoter. The human sequence, with only one base missing, is almost identical to those of the mouse and rat. This sequence contains a putative CREB site. Whether CREB binds to this site remains to be established. The third is the region from −245 to −272. There is 67% homology between human and mouse but 94% homology between mouse and rat. This region contains a putative binding site for AP-2 and Max. But the evidence that AP-2 or Max will bind to this region is still not available yet. The promoter activity of the −272 construct, which contains this region, is about 25% higher than that of the −232 construct only in IMR-32 cells but not in HepG2 cells. This suggested that this region contains cell-specific positive regulators for the IAP gene expressed in IMR-32 cells. The reporter constructs also revealed that there are positive regulators between −272 and −456 specific for HepG2 cells, because the promoter activity of the −456 construct is significantly higher than that of the −272 construct in this cell line. On the other hand, there are cell-specific negative regulators in the human IAP gene promoter. In IMR-32 cells, there are cell-specific negative reg-

\[\text{Fig. 9. Conservation of the α-Pal/NRF-1 sites in the human, mouse, and rat IAP genes.}\]

The human sequence is shown in its entirety. Bases in the sequence of mouse and rat that are identical to the human sequence are shown as dots, with only those bases that differ from the human sequence identified. The regions containing AP-2, Max, CREB, Sp1, and α-Pal/NRF-1 binding sites (underlined) are highly conserved in these three species. The missing bases are marked by −. The numbers are relative to the translation initiation codon.

\[\text{W.-T. Chang, R.-J. Chiou, C.-Y. Chen, and A.-M. Huang, unpublished results.}\]
ulators located between −272 and −456; in HepG2 cells, how-
however, there are cell-specific negative regulators located between
−730 and −1554. What the negative and positive regulators in
the IAP promoter are and whether they interact with α-Pal/NRF-1 to control the expression of the IAP gene remain
to be studied.

We conclude that the core promoter of the human IAP gene
is located between −232 and −12 nucleotides upstream of
the translation initiation codon, and that α-Pal/NRF-1 is a critical
transcription factor in the regulation of human IAP gene ex-
pression both in cell lines and primary cells. The DNA binding
activity of α-Pal/NRF-1 in different cells is an important deter-
minant of the promoter activity of the IAP gene. Understanding
the upstream signaling cascades that affect the activation of
α-Pal/NRF-1 will help to clarify the biological functions of the
IAP gene.

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REFERENCES
1. Brown, E., Hopper, L., Ho, T., and Gresham, H. (1990) J. Cell Biol. 111,
2785–2794
2. Lindberg, F. P., Gresham, H. D., Schwarz, E., and Brown, E. J. (1993) J. Cell
Biol. 123, 485–496
3. Huang, A. M., Wang, H. L., Tang, Y. P., and Lee, E. H. Y. (1998) J. Neurosci.
18, 4325–4333
4. Chang, H. P., Ma, Y. L., Wan, F. J., Tsai, L. Y., Lindberg, F. P., and Lee,
E. H. Y. (2001) Neuroscience 102, 289–296
5. Chang, H. P., Lindberg, F. P., Wang, H. L., Huang, A. M., and Lee, E. H. Y.
(1999) Learn. Mem. 6, 448–457
6. Gresham, H. D., Goodwin, J. L., Allen, P. M., Anderson, D. C., and Brown, E. J.
(1989) J. Cell Biol. 108, 1935–1943
7. Senior, R. M., Gresham, H. D., Griffin, G. L., Brown, E. J., and Chang, A. E.
(1992) J. Clin. Investig. 90, 2251–2257
8. Parkos, C. A., Colgan, S. P., Liang, T. W., Nuwit, A., Bacarra, A. E., Carnes,
D. K., and Madara, J. L. (1996) J. Cell Biol. 132, 437–450
9. Gao, A.-G., Lindberg, F. P., Finn, M. B., Blystone, S. D., Brown, E. J., and
Frazier, W. A. (1996) J. Biol. Chem. 271, 21–24
10. Wang, X.-Q., and Frazier, W. A. (1998) Mol. Biol. Cell 9, 865–874
11. Chung, J., Gao, A.-G., and Frazier, W. A. (1997) J. Biol. Chem. 272,
14740–14746
12. Torcini, M., Deckert, M., Mary, F., Bernard, G., Brown, E. J., and Bernard,
A. (1997) J. Immunol. 158, 677–684
13. Wacławieck, M., Majdic, O., Stuhi, T., Berger, M., Baumrucker, T., Knapp, W.,
and Fleck, W. F. (1997) J. Immunol. 158, 5345–5354
14. Oldenberg, P.-A., Zielesnzyk, A., Fang, Y.-F., Lagenaur, C. F., Gresham,
H. D., and Lindberg, F. P. (2000) Science 288, 2051–2054
15. Reinhold, M. I., Lindberg, F. P., Plass, D., Reynolds, S., Peters, M. G., and
Brown, E. J. (1995) J. Cell Sci. 106, 3419–3425
16. Schickel, J., Stahn, K., Zimmer, K.-P., Sudbrak, R., Sturm, T. M., Durst, M.,
Kiehnfopf, M., and Deufel, T. (2002) Biochem. Cell Biol. 80, 169–176
17. Shahein, Y. E. A., de André, D. F., and Pérez de la Lastra, J. M. (2002)
Immunology 106, 564–576
18. Aiyar, A., Xiang, Y., and Leis, J. (1996) Methods Mol. Biol. 57, 177–191
19. Jordan, M., Schallhorn, A., and Wurm, F. M. (1996) Nucleic Acids Res. 24,
596–601
20. Virbasius, C. A. Virbasius, J. V., and Scarpulla, R. C. (1993) Genes Dev. 7,
2431–2445
21. Gómez-Cuadrado, A., Martín, M., Noel, M., and Ruiz-Carrillo, A. (1995) Mol.
Cell. Biol. 15, 6670–6685
22. Dichter, M. A. (1978) Brain Res. 149, 279–293
23. Azizkhan, J. C., Jensen, D. E., Pierce, A. J., and Wade, M. (1993) Crit. Rev.
Eukaryot. Gene Expr. 3, 229–254
24. Efik, B. J. S., Chiorini, J. A., and Safer, B. (1994) J. Biol. Chem. 269,
18921–18930
25. Jacob, W. F., Silverman, T. A., Cohen, R. B., and Safer, B. (1989) J. Biol. Chem.
264, 20372–20384
26. Evans, M. J., and Scarpulla, R. C. (1990) Genes Dev. 4, 1023–1034
27. Efik, B. J. S., and Safer, B. (2000) Biochim. Biophys. Acta 1495, 51–68
28. Chalzone, F. J., Hoog, C., Teplow, D. B., Cutting, A. E., Zeller, R. W., Britten,
R. J., and Davidson, E. H. (1991) Development 112, 325–350
29. DeSimone, S. M., and White, K. (1993) Mol. Cell. Biol. 13, 3641–3649
30. Becker, T. S., Burgess, S. M., Amsterdam, A. H., Allende, M. L., and Hopkins,
N. (1998) Development 125, 4349–4357
31. Myers, S. J., Peters, J., Huang, Y., Comer, M. B., Barthel, F., and Dingledine,
R. (1998) J. Neurosci. 18, 6725–6739
32. Kumari, D., and Uddin, K. (2001) J. Biol. Chem. 276, 4357–4364
33. Silva A. J., Rosahl, T. W., Chapman, T. P., Marowitz, Z., Friedman, E.,
Frankland, P. W., Cestari, V., Ciolfi, D., Südhof, T. C., and Bourc'hluladze,
R. (1996) Curr. Biol. 6, 1509–1518
34. Gómez-Pinilla, F., So, V., and Kesslak, J. P. (2001) Brain Res. 904, 13–19
35. Gopalakrishnan, L., and Scarpulla, R. C. (1995) J. Biol. Chem. 270,
18019–18025
36. Kollmar, R., and Farnham, P. J. (1993) Proc. Soc. Exp. Biol. Med. 203,
127–139
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