MINIREVIEW

α-Fetoprotein as a Biologic Response Modifier: Relevance to Domain and Subdomain Structure (44143)

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Abstract. **In the present review, the structure of α-fetoprotein (AFP) is discussed in consideration of AFP membership and position in the albuminoid supergene family in relation to other gene family members. Ontogenetic AFP gene expression is then discussed in view of AFP mRNA presence in various tissues at different times during development. The multiple molecular forms of AFP is also presented in relation to published reports of AFP binding proteins and cell surface receptors. The review proceeds on to present AFP as a potential model of a modular/cassette protein based on sequence comparison with cleaved fragments of prohormones and biological response modifiers. Such cleaved fragments could potentially serve as peptide messengers for vascular, neuroendocrine, and digestive biological activities. Following a discussion on fibrin binding and serine proteases, AFP-cytoskeletal, extracellular matrix, and cellular adhesion interactions are considered. AFP as a carrier/transport protein based on structural relationships is further elucidated by examination of the various ligands bound to AFP and its hormone interaction. Since AFP binds heavy metals, the question is posed of whether AFP could function as an antioxidant. An analysis of transcription factors, tumor suppressors, and homeodomain proteins follows, which is interfaced with the concept of programmed cell death in light of amino acid sequence matches detected on the AFP molecule. Emphasis was naturally placed upon the homeodomain protein sequence stretches since AFP is a fetal, phase-specific protein found throughout embryogenesis, histogenesis, and organogenesis. In keeping with histogenesis, a discussion of AFP and eye lens protein development is presented. Finally, AFP sequence analysis presented in light of members of the immunoglobulin superfamily, autoimmune disorders, and various disease states culminates the review. A closing discussion then summarizes regions of presumptive matched protein identities on each of AFP's three domains.** [P.S.E.B.M. 1997, Vol 215]

The biological role(s) of α-fetoprotein (AFP) in mammals has remained an enigma for the past 30 years. Since its relationship to cancer was reported in the mid-1960s, this tumor-associated fetal protein has demonstrated clinical utility both as a tumor marker and a birth defect screening agent (1–3). Although the physicochemical and structural properties of this 70-kDa glycoprotein have been extensively described, only *in vitro* functional roles of this oncofetal protein have been ascertained to date. Such physiological properties of the oncofetal protein have encompassed mainly ligand carrier/transport functions (4) and modulation of *in vitro* immune response assays (5). Indeed, AFP has been shown to bind *in vitro* to various substances, some of which serve as ligands for members of the steroid/thyroid nuclear receptor superfamily (4–6). How-

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ever, other ligands binding to AFP (rodent and human) include bilirubin, metabolic dyes, L-tryptophan, warfarin, triazine dyes, phenylbutazone, streptomycin, phenytoin, anilinonaphthaline sulfate, heavy metals, alcohols, and drugs (7, 8). Owing to this multiplicity of carrier/transport reports, some investigators still hold that AFP is merely the fetal counterpart of serum albumin, as was previously proposed (9).

In the last decade, the growth regulatory properties of AFP have aroused interest as a result of studies involving ontogenetic and oncogenic growth in both cell cultures and animal models. A myriad of studies have now documented that AFP is capable of regulating growth in ovarian, placental, uterine, hepatic, phagocytic, bone marrow, and lymphatic cells (10–17) in addition to various neoplastic cells (i.e., MCF-7 and MTW9A breast cancer) (18). In the author's view, AFP should no longer be considered merely a fetal form of albumin only to be employed as a marker for cancer and fetal disorders. Rather, AFP should now be considered as a possible direct or indirect factor associated with the regulation of growth, differentiation, regeneration, and transformation in both ontogenetic and oncogenic growth processes.

Objectives

The present review will survey AFP as a biologic response modifier from both a structure- and a function-based viewpoint. Biologic response modifiers (BRMs), a newly named category of biomedical agents, are synthetic compounds or naturally occurring substances (i.e., proteins) that alter, temper, or modulate responses in a host organism (19). Such responses may be evident in the host at the organ, tissue, or cellular level of structural organization. Classical examples of BRMs are interferons, growth factors (proteins), peptidic hormones, cytokines, tensins, medins, kinins, and small molecules such as di- and tripeptides. BRMs cannot mount massive, substantial changes in body homeostasis, nor are they effective against overt, bulk diseases. In cancer patients, BMRs are ineffective against the total tumor body burden, although they can eradicate small numbers of tumor cells in isolated metastases throughout the body. However, when preceded by chemotherapy or radiation, BMRs are powerful growth-regulatory adjuncts in the armamentarium of tumor therapeutics. It is with this backdrop that the present treatise will address the utility of AFP as a biologic response modifier. Hard evidence interspersed with theoretical prospectives will be provided to demonstrate that a domain or a subdomain structure of AFP may determine and/or influence its role as a BRM.

A secondary objective of the present report was to review the various biological roles previously attributed to AFP and evaluate those findings in light of the more recent reports involving AFP structure and function. Owing to the recent advances in both gene cloning and amino acid sequencing, it is now possible to relate proteins and/or peptides to gene families *via* amino acid sequence matching and domain/motif structural comparison. The amino acid sequence matchings (identities and similarities) discussed below were obtained from the GCG-FASTA program from Genbank and the Swiss Protein Bank using a Xenopus-based (software) in-house computer linkage to an IBM mainframe. Inasmuch as this review focuses on the biological roles of AFP, the reader is directed to more comprehensive reviews regarding AFP structure and function (see above reviews), which cannot be detailed in the present report. It is hoped that information presented in this paper will aid investigators in search of potential new sources of biologic response modifiers and provide a foundation and rationale for proposed research in the future. As the reader will perceive, this report contains data, proposed concepts, and perspectives not previously considered in literature reviews on AFP.

Computer-Generated Data Disclaimer/Qualifier

The reader should, however, be alerted at the onset concerning the amino acid (AA) sequence identities extracted from Genbank and discussed below. Unless otherwise stated, the AFP sequence matches and identities obtained through the GCG computer software have not been subjected to biological testing. Some of the matched identities may tend to support the findings of published reports on AFP and further studies should logically be pursued. Other matched identities could be the result of biochemical coincidence, (since only 20 amino acids are present in nature) and yet some matches may have potential importance. For example, the presence of tripeptides is found randomly only once in 3000 amino acid residues. The sequence Gly-Leu-Phe is a tripeptide derived from human milk proteins that bind to human macrophages (20). Human α-fetoprotein (HAFP) has such a site on amino acids 398 to 400 located on domain 2, lending credence to this approach. Another criterion for biological significance of computer findings can readily be applied using other protein superfamily member comparisons. For AFP, the best superfamily comparison can be made using albumin as the "gold" standard. Whether domain, subdomain, or whole-molecule comparisons are made, albumin homologies tend to hold at about 30%–39% identities. However, in other gene family members such as vitamin D–binding group component (Gc) protein homologies to HAFP are lower, ranging from 20% to 30% identities. Readers can then compare and evaluate for themselves the newly found sequence identity with that of albumin and Gc protein. It should also be remembered that shorter sequence lengths, unless already published, tend to display the higher-percent identities and would naturally require closer scrutiny. Finally, it should be noted that some sequence identities may be associated with pre-, pro-, or precursor form of the protein, or a transcriptional initiation site of the matched protein. However, neither time nor space allowed for tests of individual statistical significance to be conducted among the numerous computer sequences discussed in this treatise. These sequence findings are only intended as

a guide or starting point for further investigations by the reader.

Genetic and Structural Relationships

The Albuminoid Gene Family. There is now overwhelming evidence that AFP should be classified as a member of the albuminoid gene family together with albumin (ALB), vitamin D–binding (Gc) protein (DBP) (21), and the newly discovered α-albumin (αALB) termed afamin (22). This family can be distinguished by a characteristic pattern of cysteine residues folded into loops by disulfide bridging, which reflect an overall triplicated internal domain structure (23). The ALB, AFP, and αALB genes map to human Chromosome 4 (Chromosome 5 in rodents), wherein they are localized in tandem fashion. The albuminoid multigene family encodes and translates liver-synthesized proteins that are secreted into the serum compartment to perform a variety of roles including, but not limited to, ligand/carrier transport (Table I).

The albuminoid gene translated products (proteins) display a varied array of functions in addition to carrier transport. The presence of ALB in serum constitutes a major factor in the maintenance of colloidal osmotic pressure, oxygen free-radical scavenging, esterase activity, copper-stimulated lipid peroxidation, hydrogen peroxide release, and leucocyte adherence and/or spreading (24–26). Although AFP engages in the functions described in the introduction, it may well co-contribute to and overlap with some of the roles performed by ALB. These functions will be developed more fully in later sections of this review. DBP, however, displays a truncated third domain, thereby depriving the protein of a major portion of the ligand-binding hydrophobic pocket involved in carrier/transport function (27). Nevertheless, DBP demonstrates other activities such as actin and fatty-acid binding, chemotaxis, and macrophage-activating functions closely linked to the immune response and acute-phase reactions (28). The albuminoid gene family members display significant structural similarities, homologous amino-acid sequence stretches, and similar cysteine-based secondary structures. As stated above, all four human albuminoid genes reside on Chromosome 4 within the 4q11–q22 region and most major forms contain 15 exons and 14 introns, with the exception of DBP, which displays 13 exons.

The recently discovered αALB, of unknown function, is an 87-kDa protein present in adult human and rat sera at concentrations of 20–30 μg/ml (22, 29). αALB possesses four potential glycosylation sites, reflected by an increased molecular weight from the translated mass of 66,576 daltons observed on SDS-reducing gels (Table I). αALB, also known as afamin in the human, has been proposed as a phylogenetic intermediate between the ALB and AFP genes (30). However, αALB-like molecules have now been described in nearly every vertebrate class from teleost fish to

Table I. Physicochemical Properties of the Four Protein Members of the Human Albuminoid Gene Family

Physicochemical properties	Serum albumin	α-Albumin	α-Fetoprotein	Vitamin D Gc protein
Molecular weight (kDa)	65–68	80–87	67–72	58
Number of amino acids	585	608	590	458
Peptide domains	3.0	3.0	3.0	2.6
Messenger RNA (kb)	2.1	2.3	2.2	1.7
Sedimentation coefficient (s)	4.5	NR	4.5	4.1
Serum concentration	30 mg/ml	20 μg/ml	50 μg/ml newborn	400 μg/ml
N-glycosylation sites	0.0	4.0	1.0	1.0
Percent carbohydrate (%)	<0.5	21.0	3–5	5
Lectin binding	Absent	NR	Present	Present
Chromosome location	4	4	4	4
Synthesis	Adult liver	Adult liver	Fetal liver; yolk sac; regenerating liver	Adult liver
Half-life (days)	15–16	NR	2.5	NR
Secondary structure (%)				
α-helix	47.4	NR	36.4	29.9
β-sheet	13.7	—	23.0	14.0
β-turn	26.0	—	34.0	42.0
Random coil	15.4	—	9.5	17.5
Ligand binding	Fatty acids	NR	Fatty acid	Fatty acids
	Billirubin	—	Bilirubin	Vitamin D
	Metals	—	Metals	Actin
	Steroids	—	Steroids	C5a and C5a des-Arg
	Lysolecithin	—	Dyes	

Note. These data were extracted from References 4–8. NR, not reported.

mammals including humans (7). These αALB-like molecules in the other vertebrate classes are observed as a glycosylated form of ALB, usually 72–75 kDa, in addition to the 68-kDa main serum albumin present throughout the various classes. For example, the 74-kDa glycosylated ALB of teleost fish and amphibians exhibit functions more akin to AFP than ALB (i.e., lectin, dye, and estrogen binding, as well as immunoregulatory activities). The class Aves stands as the exception to this observation in that only a nonglycosylated ALB has been reported to date. However, avian AFP has not yet been cloned and sequenced, so gene family assignments cannot be made. The AFP molecule present in mammals, and possibly birds, has been shown to be a unique and distinct moiety (albeit linked genetically) from that of αALB, which has now been reported in rodents, humans, and lower vertebrate forms (22, 29, 30). It is of particular interest that αALB is present only in postnatal and adult life, being absent from both embryonic and fetal stages of ontogeny. This observation also holds for the lower vertebrate forms with αALB-like molecules of the 74-kDa class. As discussed in a previous review of AFP phylogeny, this fetal protein (AFP) seems to be unique to only mammals and birds (7).

Ontogenetic AFP Gene Expression. Knowledge of AFP gene expression in hepatic and nonhepatic tissues of fetal and adult mammals is a prerequisite for understanding the relationship of AFP structural forms to potential functional moieties. The most detailed studies of AFP gene expression during ontogeny have been reported in fetal, postnatal, and adult rodents. Thus, the scientific literature has been largely devoid or spotty concerning AFP gene expression in mammalian species other than the rat and mouse and only now are more studies starting to appear.

The mouse (M) AFP gene is expressed in a tissue specific, temporal fashion in the cell lineages of (i) the visceral yolk sac endoderm; (ii) the fetal liver; and (iii) the fetal gastrointestinal tract (31). The MAFP messenger RNA (mRNA) comprises 20% of the total RNA in the visceral endoderm, 5% in liver, and less than 0.1% in the gut. In the postnatal mouse, AFP mRNA (assessed by RNA dot hybridization) was extremely low in adult liver and essentially absent from brain and non-AFP producing hepatomas (31, 32). These results indicated that AFP gene expression is indeed controlled at the transcriptional level. The 5′-flanking region of the MAFP gene has been found to contain a tissue-specific promoter and three upstream regulatory elements that behave as classical enhancers (33).

It is in rat gene expression that multiple AFP mRNAs have been studied in the greatest detail regarding cell type–specific expression and differential regulation (34–36). These extensive reports have now established that full-length rat AFP mRNA consists of a major form of 2.2 kilobases (kb) expressed in fetal liver and two minor variants of 1.7 and 1.5 (Table II). These latter variants and their derivatives are expressed in fetal and adult liver during hepatic regeneration, and in liver carcinogenesis (37). It should be noted at the onset that not all the AFP in mRNA transcripts produce translated proteins that are detectable; however, all forms are capable of translation *in vitro*. In fetal rat liver, the 1.7-kb mRNA is not expressed until 15 days gestation and is much less abundant than the major 2.2-kb form. The 2.2-kb mRNA encodes further translation products of 69- and 73-kDa, *in situ* hybridization showed that this form is expressed in the hepatoblasts of the fetal hepatic ductular cells and in a few nondividing hepatocytes (36, 37). It is probably the 2.2-kb translated moiety that most, if not all, of the functional studies of rat, mouse, and human AFP have been reported in the scientific literature.

In a variant rat AFP mRNA transcript, the 1.7-kb form encodes translation products of 50 and 65 kD in both fetal and adult rat liver (39, 41). After CCl_4 and galactosamine liver injury, the 2.2-kb form increases 20- to 100-fold, while the 1.7- and 1.5-kb variants change very little. Following partial hepatectomy, only small changes are observed in all the AFP RNAs; however, during carcinogenesis larger amounts of the 2.2-kb form are detected in hepatic oval cells concomitant with lowered amounts of the 1.7- and 1.4-kb mRNAs (36, 38).

Table II. Translated Proteins Encoded by Rat α-Fetoprotein (AFP) mRNAs Detected by *in Vitro* and/or *in Vivo* Expression Systems

Developmental stage	mRNA forms	Translated protein	References
Fetal liver/yolk sac (1- to 3-week) neonate	2.2 kb (major)	73 kDa, 69 kDa, (67) kDa,*	35–37
	1.7 kb	65 kDa, 59 kDa, 50 (48) kDa	
Postnatal/liver (4- to 8-week) neonate	2.2 kb (minor)	73 kDa, 69 kDa	38–40
	1.7 kb	65 kDa, 59 kDa	
	1.5 kb	50 (48) kDa	
Adult liver/kidney (8-week and older) neonate	2.2 kb (trace)	73 kDa, 69 kDa	41, 42
	1.7 kb	65 kDa, 59 kDa	
	1.5 kb	50 (48) kDa	
	1.35 kb	44 (37) kDa	
Hepatoma liver/regeneration	2.2 kb (major)	73 kDa, 69 kDa	36, 37, 39, 40
	1.7 kb	65 kDa	

Note. Hepatoma AFP mRNA is most similar to the fetal type. Parentheses indicate the major, minor, or trace form found in all AFP mRNA populations at that developmental stage of ontogeny.
* Differs by glycosylation amounts.

The adult rat liver thus contains three AFP mRNAs consisting of the 2.2-, 1.7-, and the 1.5-kb forms. The 1.7- and 1.5-kb forms of Chou (40) correspond to the 1.4- and 1.0-kb forms described by Lemire and Fausto (36). The fetal rat liver contains mainly the 2.2-kb mRNA, which decreases to a very low level at the fifth week following birth. The 1.7- and the 1.5-kb forms are observed by the third and fourth postnatal week and represent only 0.4% of the total mRNA compared with the 18-day fetal liver (40, 41). The latter two adult AFP mRNA expressions can be inhibited by methyl-isobutyl-xanthine (MIX) while the fetal 2.2-kD form is stimulated by this compound. These data suggest that, in the rat, the fetal versus the adult AFP transcripts are both developmentally and differentially regulated. Thus, it is possible that cAMP (MIX-responsive) is one of the regulators that controls the differential expression of the AFP gene during development. Of course, other regulators of AFP gene expression, such as glucocorticoid, *fos* and *jun,* and retinoic acid, have also been reported (42–44).

The adult rat liver mRNA that Chou *et al.* (41) reported was another variant AFP mRNA of 1.35 kb in addition to the 1.7- and 1.5-kb found in adult rat liver and kidney. The 1.35-kb mRNA contains an open reading frame of 325 AA and directs the synthesis of a nonglycosylated polypeptide of 37 kDa. However, the variant AFP protein is devoid of the first third of the amino-terminal side of the fetal AFP molecule and begins at amino acid 278 onward to the carboxyl terminus. Since the most specific antibodies to AFP are classically directed against the first domain, most anti-rat AFP antibodies would precipitate only 10% or less of the 37-kDa variant. In the future, monoclonal and/or polyclonal antibodies specific to the 37-kDa variant would have to be produced in order to more clearly identify this variant AFP in adult rats (see below). Detection and analysis of the variant forms of HAFP might have potential clinical utility.

Tissue culture COS cells infected with the 1.35-kb cDNA yielded an intracellular polypeptide of 37 kDa lacking a signal peptide, which was retained in the cells as a nonsecretory form. AFP nonsecretory forms have previously been reported in both rat uterine and cancer cells (45, 46). In addition to the rat liver and kidney, the 1.35-kb variant was detected in the rat uterus but not in the brain, gut, heart, ovary, spleen, stomach, testes, or placenta. The expression of the 1.35-kb AFP variant in a variety of rat tissues and cell lines raises the possibility that a similar transcript might be expressed in other species such as human, since a nonsecreted bound form of AFP has been reported in human breast cancer cytosols (47). It is highly probable that adult human AFP assayed by means of RIA or EIA may well be the fetal 2.2-kb transcript, while the variant translated products are virtually undetectable using communically available antisera.

It is germane to the present discussion that messenger RNA for human AFP has been partially purified from human testicular embryonal carcinomas that have been inserted into nude mice (48). Following hybridization studies with DNA complementary to mouse AFP mRNA, a plasmid containing 900 nucleotides was further characterized by (i) hybrid arrest of AFP mRNA translation; (ii) restriction endonuclease mapping; and (iii) partial nucleotide sequence analysis. These investigators found that the AFP complementary DNA (cDNA) sequence corresponded more closely to the 3′ end (carboxyl terminus) of HAFP mRNA. Assuming that human AFP mRNA is 2200 bp long, the variant isolate represented about 40% of the HAFP mRNA sequence from the carboxyl terminal end. Thus, the entire first domain and 20% of the second domain of RAFP are missing in this moiety. This mRNA transcript seems to resemble the 1.35-kb variant described above in the adult rat liver and observed in rat carcinogenesis (41, 49).

An equivalent human variant AFP form, if one exists, might well reside in an intracellular site either free or complexed to a macromolecule as discussed in the next section. The function of such a postulated protein might involve an autocrine cell-regulatory function since it is retained in a nonsecretory form. Thus, the 5- to 10-ng/ml AFP detected in the sera of the normal adult human probably represents only the 2.2-kb translated mRNA transcript, while none of the human AFP variants, if they exist as described above, are even measurable with present methodology.

Molecular Forms of AFP. *Free and bound forms.* Reports of the presence of AFP in both free and bound molecular forms have emerged concerning both mammalian sera and tissue cytosols (Table III). Circulating ''free'' AFP has long been recognized as an immunoreactive form with a molecular weight of 70 kDa (4), whereas the bound form of AFP is described as a nonimmunoreactive form with a total molecular weight frequently approaching 200kDa (53, 54). This larger molecular weight is attributed to a protein moiety, that binds to the AFP, masks its antigenic epitopes, and can be displaced either by high salt concentrations (0.4 *M* KCl) or by various serine protease inhibitors (PI) and/or substrates (58, 60) (see section entitled, ''AFP, Fibrin Binding, and Serine Proteases,'' below). In blood, a bound form of AFP has been detected in specimens from both pathological (malignant and nonmalignant) and normal human sera migrating in the β- and γ-electrophoretic regions and sometimes detectable as an immunoglobulin-G– or M–like complex (55, 57). AFP circulating in the bound form has also been reported in the sera of patients afflicted with breast cancer (56).

Possible candidates for the protein moieties that bind to AFP (aside from IgG or IgM) could include cytoskeletal and extracellular matrix proteins and/or albumin-binding proteins (43 kDa) similar to osteonectin, which is secreted by endothelial cells and circulates in blood bound to serum albumin (62, 63). Proteins (receptors) binding albumin have frequently been shown to display a similar affinity for AFP (50). Worthy of note is that plasma-borne cytoskeletal-related moieties could qualify as AFP binding components detected as a complex in the β, γ-region of the electrophoretic spectrum. One such protein (43 kDa) is associated with

Table III. Multiple Binding Proteins for Rodent and Human α-Fetoprotein (AFP) Categorized according to Their Extra- and Intracellular and Membrane Localization Sites

Compartment location	Molecular form	Affinity constant (*k*D)	Binding site #	References
Cell surface membrane				
Glycopeptide (Raji cell)	18 kDa	5×10^{-8}	ND	50
Glycopeptide (Raji cell)	31 kDa	5×10^{-8}	ND	50
Glycoprotein (monocyte/phagocyte)	62, 65 kDa	5×10^{-11}	49	
		5×10^{-7}	7,800	51
MCF-7 breast cancer cells	42 kDa	5×10^{-9}	2,000	
		1×10^{-8}	135,000	52
Y-1 adrenal tumor (mouse)	ND	5×10^{-9}	350,000	AUD
PHA lymphocytes	ND	3×10^{-7}	25,000	
	ND	2×10^{-6}	ND	
		Tissue/organ	Normal (N)/ pathologic (P)	
Extracellular biological fluid				
Plasma/sera	β-, γ-component	Blood	N,P	53–55
Sera	KCl-Extract	Breast	Tumor	56
Sera	β-, γ-	Liver	Cirrhosis	54
Immunoglobulin EP region	IgM, 200 kDa	Liver	Cirrhosis	55
Immunoglobulin EP region	IgG component	Systemic	Lupus erythematosis	57
Fetal fluid	Protease inhibition extract	Amniotic fluid	Normal	58
Intracellular biological fluid				
Human cytosol	KCl, PI extract	Uterus Breast Ovary	Cancer	45, 58
Rat cytosol	E2-receptor associated	Uterus	Normal	59
Rat cytosol	KCl extract	Uterus	Normal	45
MCF-7 cytosol	5×10^{-8} 75 kDa	Breast	Cancer	60
Human cytosol	62 kDa 67 kDa	Breast	Cancer	52
Human cytosol	185 kDa associated*	Breast	Cancer	61

Note. KCl, potassium chloride; PI, protease inhibitor; EP, electrophoretic; AUD, author's unpublished data; ND, not determined.
* AFP does not bind this component, it is only associated with it.

the acetylcholine receptor (AchR) and is an actin-binding protein (64). Past studies have demonstrated that the AchR is a cell-surface multiprotein complex anchored to 43-kDa globular proteins that cross-link the receptor to cytoskeletal actin, ankyrin, or spectrin filaments (64, 65).

*AFP-binding proteins. **Vascular-lining components.*** It has been reported that AFP is capable of complexing, at high-affinity binding sites, to a variety of polypeptides both in the intravascular and extravascular compartments. Interestingly, 18-, 31-, and 60-kDa cell-surface receptors have been previously identified as binding to albumin (66–68). Reports have demonstrated that AFP shares binding specificities with albumin for the 18- and 31-kDa binding proteins (50). The 60-kDa (gp 60) protein is known to be an endothelial cell surface sialoglycoprotein, which binds albumin on continuously lined endothelium of many nonbrain tissues (heart, lung, epididymis, etc.), whereas the 18- and 31-kDa proteins represent scavenger receptors on all other tissue endothelium regardless of location (68, 69). The latter two scavenger receptors, which also have affinity for AFP, have been reported to bind chemically modified or altered albumin 1000 times more strongly than intact, native albumin (66, 67). This scavenger receptor-mediated endocytosis has been found to funnel denatured protein into a catabolic pathway leading to lysosomal degradation and exocytosis of degraded protein residues. This metabolic pathway for AFP has been discussed in a previous report (70).

AFP membrane receptors. The albumin binding 60-kDa receptor (see above) lining the microvascular endothelium might resemble a 62- to 67-kDa cell-membrane receptor reported as specific for AFP (51, 52). The 60-kDa albumin receptor appears to be a glycophorin-like sialoglycoprotein that is believed to create a selectively permeable barrier that limits the transendothelial passage of many molecules through endothelium (69). Glycophorin-like proteins, once considered to be exclusively erythrocyte associated, have now been identified on a variety of cell types and partially contribute to the MNS blood group erythrocyte family of antigens (71). Autoantibodies against the glycophorin-like proteins are known to exacerbate various forms of autoimmune hemolytic anemias. Since albumins have already been reported to bind to the surface of red blood

cells (RBCs) (72), it is noteworthy that human AFP has been demonstrated to inhibit isoagglutinins against the RBC antigens of the AB blood groups (73). This agglutination inhibition could be attributed to a competitive binding of AFP molecules with autoantibodies directed against a glycoprotein-like protein antigen on the RBC surface. Endothelial cells are also known to secrete an albumin-binding molecule (osteonectin, SPARC), which can circulate in the blood bound to albumin (74). This 43-kDa protein, or actin itself, could serve as possible candidates for the elusive serum AFP-BP first described by Norgaard-Petersen (53). A discussion of AFP:cytoskeletal and extracellular matrix interactions is considered in subsequent sections of this review. A review of AFP-binding proteins has previously been addressed (75).

AFP as a Modular/Cassette Protein. Many proteins are now known to serve as precursor molecules which contain multiple modular sequences or cassette segments generated by proteolytic processing to produce smaller biologically active peptides. Such precursor molecules (prefixed as pre- and proproteins) are often recognized as proenzymes or prohormones, which contain multiple sites with two or more adjacent basic residues that serve as potential proteolytic cleavage sites. The classic example of modular processing and cleavage is the pro-opiomelanocortin (POMC) precursor protein found in the vertebrate pituitary (corticotrophs and melanotrophs) and hypothalamus. The POMC precursor is proteolytically clipped at various arginine-lysine sites to produce a host of biologically active peptide hormones such as γ-α-and β-melanocyte–stimulating hormones (MSHs), β-endorphin, β-lipotrophic hormone (β-LPH), and ACTH (76). Another example would include the epidermal growth factor–like (FGF) proteins, which display module domains or cassettes with similar recognizable EGF sequences found in a wide variety of different proteins (77). Such modules are known to be present in a broad spectrum of proteins including those associated with blood coagulation, fibrinolysis, neural development, and cell adhesion. These domain or subdomain modules, comprising about 45 residues, seem to be involved in the mediation of protein-protein interactions. Some of the more diverse mosaic proteins that have been found to contain EGF modules are tissue growth factor–α, thrombomodulin, L-selectin, Neu differentiation factor, neurexin, protein C, tissue plasminogen activator, blood factors VII, IX, X, and XII, and complement factors Clr, Cls, and C6 (76, 77).

In many cases, a single, large polypeptide has been found to serve as the precursor prohormone for several biologically active peptide fragments that can exert different functions in the gastrointestinal, endocrine, cardiovascular, and nervous systems. The peptidic and amino acid fragments cleaved from the "mother" protein may have a variety of roles including biological response antagonism or agonism, feedback control, and hormone modulation. Gastric hormone (GRH) and cholecystokinin (CCK) belong to the same family of peptides and their C-terminal pentapeptides are identical (76). Gastrin stimulates gastric acid secretion, smooth-muscle contraction, and exerts trophic action on various gut target tissues. CCK is the major hormonal stimulator of gallbladder function and pancreatic exocrine secretion. From both the GRH and CCK precursors, multiple fragments have been found *in vivo* representing proteolytic cleavage remnants with potent biological activities. Proglycogen also gives rise to glicentin-related peptides that inhibit glucose-induced insulin release (78). The opiod endorphin peptides, such as Met and Leu enkephalin, are derived from ACTH, LPH, and POMC as described above. Tufsin, a cytophilic blood factor, represents a proteolytic fragment from amino acids (AA) 289–292 of immunoglobulin-G (79). Rigin, a phagocytosis-stimulating factor, is likewise derived from amino acids 341–344 of IgG. Substance P, a bioactive peptide derived from tachykinin, is capable of smooth muscle contraction, reduction of blood pressure, and stimulation of secretory cells (80). Finally, enzyme inhibitors that can be cleaved from acidic proteases of the renin-angiotensin system can mimic the "transition state" of the enzyme-catalyzed reactions under study (76).

In the albuminoid gene family, it is well known that human serum albumin is produced as a proprotein and includes intermediate precursor forms that could gain entrance to the plasma (81). Truncated forms of ALB have also been reported to exist as mini- and microalbumin forms (82). In addition, peptides derived from immunologic processing of serum ALB produce an epitopic 22-AA fragment, which binds to human MHC class II DRw11 molecules present on antigen-processing cells *in vivo* (83). Coincidentally, an albumin allergenic protein fragment isolated from dog salivary glands shares a 57% sequence identity with HAFP (domain 2) extending over 14 amino acids. Serum ALB is also enzymatically cleaved during immune macrophage processing to multiple tripeptides such as Glu-Lys-Thr, Asn-Ala-Glu, Gln-Ala-Ala, and Leu-Lys-Ser (84). Although AFP has been shown to display mRNA forms that produce truncated molecules (see "Ontogenetic AFP Gene Expression" above), a methodical study of peptides enzymatically cleaved from native AFP has not been reported aside from peptide fragment preparations employed for mass-spectrometric analysis (85).

Plasma proteins, such as ALB and AFP, could possibly serve as circulating reservoirs of biological response-modifying peptide fragments (86). Limited proteolysis of protein substrates appears to be a general mechanism for the generation of various regulatory peptides in the circulation, lymphatics, and intracellular spaces. Vascular homeostasis provides the best known examples as displayed in fragment generation of angiotensin I from angiotensinogen by renin and the generation of bradykinin from kininogen by kallikrein (87). Neurotensin and neuromedin-like fragments can both be produced from plasma by the treatment with pepsin (88, 89). These peptides are capable of modifying

biological responses in the endocrine, cardiovascular, digestive, reticulendothelial, and central nervous systems. Such peptides can be isolated from pepsin-treated human, bovine, canine, and rattine plasma (89, 90).

Pepsin-isolated peptides have shown amino acid sequence homology to both ALB and AFP. As shown in Table IV, both human and mouse AFP show striking sequence homologies with a neurotensin-related peptide (i.e., kinetensin) starting at HAFP AA sequences 145 and 339. Furthermore, the apparent similarities between these AFP sequences and neurotensin, neuromedin, and related peptides are obvious. Six of nine residues in the first domain of (amino acids HAFP 146–154) are identical to the neurotensin-related peptide, while four of nine are identical in the second domain of HAFP (amino acids 339–447). The HAFP third domain is less significant in AA identity; however, amino acid similarity is observed in five of nine residues. Mouse AFP is less dramatic, showing mainly amino acid similarities rather than identical sequences. Although the sequence homologies of AFP with angiotensin, xenopsin, and neuromedin are less pronounced, the obvious relationship of basic residues (Lys, Arg, His) to proline relationship is maintained. In neurotensin, it is the Arg adjacent to the His-Pro and the terminal Leu that are important for receptor binding, while the Pro-Tyr doublet is required for intrinsic activity (89, 91). Both mouse and human AFP display the basic residues-to-proline patterns, which could influence receptor binding phenomena. These present observations suggest a possible role of albuminoid gene family proteins (AFP) as a potential source of circulating precursors of peptide messengers for vascular, neuroendocrine, and digestive biological activities. As discussed in the remainder of this report, amino acid sequence identities from a multitude of proteins detected on short stretches of AFP lend credence to a modular/cassette hypothesis.

The concept can then be extended in that AFP might consist of multiple subdomain modules and/or amino acid cassettes that, following proteolytic cleavage and/or processing, display biological modifying activities of their own. This hypothesis has been tested by employing synthetic peptides modeled from amino acid sequences actually present in the human AFP molecule. Studies from the author's laboratory have revealed that a synthetic peptide, fashioned after the carboxyl-terminal side of the major hydrophobic steroid binding pocket, displayed estrogen-sensitive regulatory growth activity (92). This 34-mer peptide demonstrated antiestrogenic activity in both a uterotrophic *in vivo* bioassay and an *in vitro* estrogen-induced focus formation in MCF-7 human breast cancer cultures. The AFP-derived peptide further inhibited an estrogen-dependent increase in uterine-derived thrombin and tissue factor as determined by an enzymatic esterase assay. Although the mechanism of the AFP-derived peptide inhibition of estrogen-dependent growth remains to be determined, the uncoupling of signal-transduced early/immediate gene induction looms as a viable candidate. This published report should serve as prototype for future studies for investigators searching for biologically active amino acid sequence stretches on the AFP molecule as postulated in this report (see also "Interactions of AFP with Hormones and Growth Factors" below).

Pericellular Structure/Function Relationships

AFP, Fibrin Binding, and Serine Proteases. Human tissue–type plasminogen activator (TPA) is a serine protease enzyme responsible for dissolving fibrinogen in blood clots (93). Interestingly, an amino acid sequence site detected on HAFP domain 1, located by computer Genbank analysis, showed a 35% sequence identity over 20 residues to γ-fibrinogen. Both plasminogen and TPA are bound by

Table IV. A Human Kinetensin Amino Acid Sequence and Related Peptides Compared with Segments from Human and Mouse α-Fetoproteins

Protein/peptide	Amino acid three-letter code								
A. Human α-fetoprotein (HAFP)									
Angiotensin	Arg	**Val**	Tyr	Ile	**His**	**Pro**	Phe	His	**Leu**
Xenopsin	(–)	Glu	Gly	Lys	Arg	**Pro**	Trp	**Ile**	**Leu**
Neuromedin	(–)	(–)	(–)	Lys	Ile	**Pro**	**Tyr**	**Ile**	**Leu**
Neurotensin	Asn	Lys	Pro	**Arg**	Arg	**Pro**	**Tyr**	**Ile**	**Leu**
Kinetensin	Ile	Ala	**Arg**	**Arg**	**His**	**Pro**	**Tyr**	Phe	**Leu**
HAFP (145–154)	Ile	Ala	**Arg**	**Arg**	**His**	**Pro**	Phe	Leu	Tyr
HAFP (339–347)	Tyr	Ser	**Arg**	**Arg**	**His**	**Pro**	Gln	Leu	Ala
HAFP (536–544)	Leu	**Val**	Lys	GLn	Lys	**Pro**	Gln	Ile	Thr
B. Mouse α-fetoprotein (MAFP)									
Neuromedin	Asn	Lys	Pro	**Arg**	Arg	**Pro**	Tyr	Ile	**Leu**
Kinetensin	Ile	Ala	**Arg**	**Arg**	**His**	**Pro**	Tyr	Phe	**Leu**
MAFP (141–150)	Val	**Ser**	**Arg**	**Arg**	Asn	**Pro**	Phe	Met	Tyr
MAFP (334–343)	Tyr	**Ser**	**Arg**	Thr	**His**	**Pro**	Asn	Leu	Pro
MAFP (531–540)	Leu	Val	Lys	Gln	Lys	**Pro**	Glu	Leu	Thr

Note. Identical amino acid residues are bold-faced; similar amino acid residues are underlined. Data were extracted and expanded from References 89 and 90.

the fibrin polymer in clots to facilitate enzyme: substrate binding alignment (Table V). It has further been reported that human TPA residues 72–110 display significant sequence homology corresponding to the last 39 residues at the carboxyl terminus (domain 3) of HAFP (552–590) (94). A portion of this segment on TPA (AA 44–90) overlaps a region that was previously shown to be related to the epidermal growth factor motif module (95), as discussed above. TPA identity sites have also been detected on HAFP domains 2 and 1. The domain 2 site displayed 33% identity over 15 amino acids, while the domain 1 region showed 32% extending over 19 residues. The NH_2-terminal portion of TPA (AA 6-43) contains the fibrin-binding finger-domains for the serine protease/substrate contact and this region resembles HAFP residues 426–453 in the middle of the third domain of the AFP molecule (Table V, A5).

Interestingly, this region is also known to be the major hydrophobic binding pocket of HAFP, which corresponds to the estrogen-binding site of rodent AFP (96). Residues crucial for estrogen binding on rat AFP are amino acids 428, 430, 433, 434, and 435 (97). Thus, the estrogen binding pocket of RAFP may overlap or at least contain a portion of a site that could function as a serine protease site for binding substrate. If this were the case, AFP might bind serine protease substrate and/or inhibitors, but not cleave them as was previously proposed (98). Indeed, a search of the literature reveals a series of reports that seem to confirm this thesis.

Studies in the latter 1970s had predicted that steroid hormone-binding proteins contain spatially adjacent histidine and serine residues, which could aid in binding protease inhibitors/substrates and also regulate steroid hormone binding (99). Subsequent reports had showed that protease inhibitors (tosyl-lysine-chloro-methyl ketone) and substrates (tryptophan methyl ester) eliminated specific binding of steroids to their respective nuclear receptors as well as estrogen binding to rat AFP (100, 101). Further studies revealed a specific interaction between RAFP and chymotrypsin substrates indicating structural binding-site similarities between AFP and the substrate-binding site on chymotrypsin. Thus, RAFP binding to estrone was found to be inhibited by tryptophan methyl ester, and the reaction was stereospecific and pH dependent (102, 103). These investigators further demonstrated that tryptophan-nitrophenyl esters had an even greater binding affinity to RAFP and that the reaction was reversible and competitive with estrogen binding. The authors concluded that AFP contained a binding site for tryptophan esters that contained a nucleophilic group(s) with high affinity for esters and appeared to overlap or lie adjacent to the estrogen-binding site. It is of special interest to the present review that the human estrogen receptor (ER) also has a similar site, which regulates estrogen binding and which structurally resembles the substrate-binding site of chymotrypsin (104). More recently, these studies were extended showing that estradiol receptor (ER) transformation was due to an effect of serine protease intrinsic activity of the ER (105) and that aprotinin (a protease inhibitor) can inhibit estrogen binding to that nuclear receptor (106).

In keeping with the concept that AFP contains serine protease-like motifs that bind protease inhibitors/substrates, studies were also pursued in the author's laboratory. It was first demonstrated by radioimmunoassay that "bound" AFP in human serum and cytosols could be converted to a "free" form (increased amounts) by preincubation with a variety of trypsin substrate and/or inhibitors (58) comparable to previous studies employing high salt concentrations (60). It was further observed that mouse AFP could be bound to benzaminadine-linked sepharose columns but not trypsin-linked columns, suggesting that AFP was capable of binding serine protease substrates. Further studies showed that ^{125}I-HAFP could bind to benzaminadine-linked beads and then be specifically purged from these beads by micro-

Table V. Potential Fibrin-Associated Proteins, Serine Proteases, and Inhibitor/Substrate Displaying Human α-Fetoprotein (AFP) Amino Acid Sequence Identities

Protein name/type	Amino acid sequence identity (%)	Amino acid length (≠)	Human AFP domain
A. Fibrin-associated and serine proteases			
1. γ-Fibrinogen	35	20	1
2. Tissue plasminogen activator (site I)	32	19	1
3. Tissue plasminogen factor (site II)	33	15	2
4. Human proteinase	34	23	2
5. Acetylcholinesterase	30	27	3
6. Carboxypeptidase (metalloproteinase)	38	29	3
B. Serine protease inhibitor/substrates			
1. Anti-thrombin III	41	34	1
2. Heparin co-factor (HCF2) serpin	45	20	2
3. Inter-α-trypsin inhibitor	38	24	2
4. Ovalbumin	38	16	2
5. Kallistatin serine proteinase inhibitor	29	25	3

Note. Only sequences showing greater than 30% identity and 15-AA length are listed.

molar concentrations of arginine (a specific substrate for serine proteases) in a competitive manner. Purified mouse AFP was also observed (58) to compete with inter-α-trypsin inhibitor (IATI) for elastase; curiously, a sequence identity site for IATI resides on HAFP domain 2 (38% identity, 24 AA in length). It was further found that an AFP-like protein complex with demonstrable electrophoretic γ mobility was present in both sera and in tissue cytosols from liver and reproductive tissues (58). It was concluded that both human and rodent AFP appeared to contain a binding site(s) with an affinity for protease substrate/inhibitors consistent with the reports described above.

The Actin Cytoskeleton, Intermediate Filaments, and Microtubules. Actin, an abundant intracellular protein, is known to circulate at micromolar concentrations in peripheral blood (107). In mammals experiencing diverse forms of tissue injury, actin is released from dying cells and may be entrapped in fibrin clots adjacent to or directly at tissue damage sites. Actin has been reported to be a noncompetitive inhibitor of the clot-dissolving enzyme, plasmin (108). Adult plasma reportedly contains two high-affinity actin-binding proteins (i.e., vitamin D-binding protein [Gc globulin] and plasma gelsolin), which serve as actin-sequestering agents to protect against microvascular damage. Thymosin also serves as a major actin-sequestering protein in the cell (109). Within the cell itself, actin is a dominant, abundant cytoskeletal protein existing in monomeric, filamentous, and protein-complexed forms. Profilin, also a major intracellular protein, binds monomeric G-actin to constitute an intracellular pool of nonfilamentous actin (107). The profilin-actin complex is called profilactin and serves as an intracellular repository of available actin. Previous reports have demonstrated that actin can inhibit plasmin's hydrolysis of substrate, suggesting that accessible lysine residues of actin interact with the kringle fingers (lysine-binding regions) of the plasmin molecule (108). The authors of the latter study suggested that extracellular-released actin may modulate plasmin-dependent biological responses.

The actin binding site on vitamin D–binding protein (DBP) has been mapped to AAs Asp 385 to Thr 400 at the carboxy-terminal region of domain 2 by mutation/deletion manipulation coupled with monoclonal-antibody targeting studies (110). These studies and others (110, 111) further noted that DBP undergoes significant conformational changes following actin binding regarding fatty acid displacement and physicochemical properties. As noted in Table VI, HAFP shows high amino acid sequence homology at the amino acids 376–391 when compared with the actin-binding site on DBP. These findings together with the sequence homology of the AbL proteins shown below (Table VI, bottom), prompted studies in the author's laboratory to further elucidate these observations. Constant amounts of ^{125}I-HAFP were titrated against 10-fold dilutions of purified actin; following incubation, anti-AFP coated beads were added to the mixture to capture free radiolabeled AFP. These preliminary studies revealed that purified nondiluted actin bound 50% of the radiolabeled AFP, while a 1000-fold dilution of actin bound only 13% of the available counts. A further challenge of mouse AFP preincubated with actin in an aggregation/polymerization assay also indicated a 50% reduction in the formation of polymerized actin. Although both studies have yet to rule out plasma DBP and gelsolin contamination, these observations serve to underpin the amino acid sequence homology data.

If AFP did possess an actin-binding and/or neutralization role, then other physiological and pathological aspects

Table VI. Sequence Comparisons

A. Between the actin-binding site of the human vitamin D–binding protein (HDBP) and mouse/human α-fetoprotein (AFP) segments

HDBP (≠185)	**Asp**	**Lys**	**Gly**	Gln	Glu	—	**Leu**	Cys	Ala
HAFP (≠376)	**Asp**	**Lys**	**Gly**	**Glu**	**Glu**	**Glu**	**Leu**	—	Gln
MAFP (≠371)	**Asp**	Asn	Leu	**Glu**	**Glu**	**Glu**	**Leu**	—	Gln
HDBP (≠193)	Asp	Tyr	Ser	**Glu**	Asn	Thr	Phe	Thr	
HAFP (≠384)	**Lys**	Tyr	**Ile**	Gln	Glu	Ser	Gln	Ala	
MAFP (≠379)	**Lys**	His	**Ile**	**Glu**	Glu	Ser	Gln	Ala	

B. Between the c-Abl actin-binding proto-oncogene products (nonreceptor tyrosine kinases) and mouse/human α-fetoprotein segments

MAFP (≠374)	Gly	Cys	Gln	**Asp**	**Asn**	Gly	—	Glu	Glu	—	Glu	
HAFP (≠369)	Glu	Cys	Gln	**Asp**	**Lys**	**Leu**	—	Glu	Glu	—	Glu	
Mc-Abl	**Gln**	**Asp**	Phe	Ser	**Lys**	**Leu**	**Leu**	**Ser**	—	—	—	
Hc-Abl	**Gln**	**Asp**	Phe	Ser	**Lys**	**Leu**	**Leu**	**Ser**	—	—	—	
Arg	Pro	Val	Leu	Asn	**Asn**	**Leu**	**Leu**	**Ser**	—	—	—	
D-Abl	**Gln**	**Asp**	Asn	Glu	Arg	**Leu**	Val	Ala	**Glu**	Val	Gly	
MAFP	Leu	Gln	—	**Lys**	His	**Ile**	Glu	Glu	Ser	Gln	Ala	Leu/Lys
HAFP	Leu	Gln	—	**Lys**	Tyr	**Ile**	Gln	Glu	Ser	Gln	Ala	Leu/Lys
Mc-Abl	—	**Ser**	**Val**	**Lys**	Glu	**Ile**	**Ser**	Asp	**Ile**	**Val**	Arg	**Arg**
Hc-Abl	—	**Ser**	**Val**	**Lys**	Glu	**Ile**	**Ser**	Asp	**Ile**	**Val**	Gln	**Arg**
Arg	—	Cys	**Val**	Gln	Glu	**Ile**	**Ser**	Asp	Val	**Val**	Gln	**Arg**
D-Abl	Gln	**Ser**	Leu	Arg	Gln	**Ile**	**Ser**	Asn	Ala	Leu	Asn	**Arg**

Note. Boldface indicates amino acid identical sequences; underlined residues signify amino acid similarity. M, Mouse alpha-fetoprotein; Mc-Abl, Murine Abl actin-binding domain AAs 1065–1097; HC-Abl, Human Abl actin-binding domain; Arg, Human arginine; D-Abl, Drosophila Abl actin-binding protein.

might be tenable. For example, an actin-binding function contributes to cell transformation in the Philadelphia-chromosome–positive human leukemias (112). The BCR-Abl oncoprotein complex (Table VI), if prevented from binding F-actin, showed a reduced ability to transform Rat-1 fibroblasts, which mimic the leukemia cell transformation. While BCR activates the Abl tyrosine kinase, the presence of both actin binding and a DNA-binding domain the Abl proto-oncogene suggests that the protein may be capable of relaying a signal from the cytoskeleton to the genome in a single step. The actin-binding state of the oncogene transformation induces a redistribution of dispersed intracellular F-actin into punctate aggregates surrounding the cell nucleus (112). This process has been proposed by these authors to interfere with the normal signal transduction pathways by disrupting interactions between the actin cytoskeleton and growth factor/cell adhesion receptors in the plasma membrane. It would not be unreasonable to implicate AFP in such actin-coupled growth regulatory signal pathways in view of the association of AFP with growth, differentiation, regeneration, cell transformations, and cellular adhesion discussed below.

HAFP appears to further display short amino acid segment homologies (identities) to the cytoskeletal proteins that could aid in the eventual elucidation of function based on structural analysis (Table VII). These proteins include the actin-associated filaments, the motor and microfilaments, and the intermediate filaments (113). These stretches of HAFP amino acids, extracted from Genbank, are displayed in Table VII. After partitioning the cytoskeletal proteins into their three classes, certain trends and/or patterns emerged. First, the actin-associated proteins' identities appear to be clustered on domain 2 of HAFP. In contrast, the microfibril and motor filament (myosin) amino acid identities are amassed largely on HAFP domain 1. Finally, the intermediate-filament protein homologies are more widely distributed, being predominantly evident on domains 1 and 2. It is of interest that amino acid identity sequences of two anchor proteins namely, ankyrin and catenin, were both localized at the carboxy-terminal domain of HAFP. These computer data findings would suggest a trend in which certain areas on the HAFP molecule might conceivably provide a dimerization interface for interaction with actin, myosin, and the intermediate filaments. Such surface interactions with actin/myosin might implicate muscle contraction, cytokinesis, cytoplasmic streaming, ameboid motion, and cross-linking with the intermediate filaments. The synthesis of AFP has previously been reported in neonatal rat skin implants (114). Also, the elevated AFP levels reported in infants with functional and dystrophic epidermolysis bullosa, aplasia cutis congenita, and epidermolysis bullosa letalis (115–117) attest to a possible linkage between AFP and the intermediate filaments. In fact, it has been suggested that AFP screening might obviate the need for fetal skin sampling in the prenatal diagnosis of these disorders.

Extracellular Matrix and Cell Adhesion Molecules. The major classes of molecules that regulate cellular development and physiology include growth and differentiation factors, cell adhesion molecules, and components of the extracellular matrix (ECM). The ECM molecules, once thought the least interesting, have now been shown to influence the same major phases of development and cell function as those regulated by growth and differentiation factors (118). Components of the ECM and their receptors interact with virtually all cell types (including the nervous system) in embryonic and adult organisms (Table VIII). Thus, the ECM presently is defined to include essentially all secreted molecules that are immobilized outside of cells. The major ECM constituent members encompass the collagens, elastins, laminins, surface glycoproteins, and the proteoglycans. The functions of these diverse molecules include inhibition of neurite outgrowth, cell migration during embryogenesis, modulation of synaptic transmission, antiadhesive activities, growth factor interaction,

Table VII. Potential Microfibril Intermediate Filaments, and Actin-Associated Filament Proteins Displaying Human α-Fetoprotein Sequence Identities

Protein	Amino acid sequence identity (%)	Amino acid length (≠)	Human AFP domain
A. Microfibrils and motor filaments			
Transducin	45	20	1
Kinesin	28	29	1
Myosin (α-type)	33	18	1
B. Intermediate filaments			
Cytokeratin	28	29	1
Profilaggrin	35	26	1
Neurofilaments	33	15	2
C. Actin-related filaments			
Actin F-form	44	16	2
Spectrin	44	18	2
Ankyrin	32	22	2
Tropomyosin	41	22	2
Twitchin	39	31	2

Note. Only sequences showing at least approximately 30% identity and 15-AA length are listed.

Table VIII. Potential Extracellular Matrix (ECM) Proteins Displaying Humans α-Fetoprotein Amino Acid Sequence Identities

Protein name/type	Amino acid sequence identity (%)	Amino acid length (#)	Human AFP Domain
Fibulin	30	28	1
Synedecan	35	17	1
Fibronectin	44	16	2
Integrin	44	23	2
von Willebrand factor	47	17	2
Perlecan	36	22	2
Collagen I	43	22	2
Collagen IV	43	16	2
Collagen VI	47	15	2

Note. Only sequences showing greater than 30% identity and 15-AA length are listed.

ECM anchoring, binding of proteases or protease inhibitors, initiation of intracellular signaling cascades, signal transduction *via* the cytoskeleton, and pH modulation of Na^+/H^+ antiporter activity (118). It may be of interest that HAFP domain 2 displays a sequence identity site (44% identity over 18 amino acid lengths) to a sodium antiporter system.

The cell adhesion molecules (CAMs) mediate cell-cell and cell-extracellular matrix (ECM) interactions during embryonic development, maintenance of adult tissue architecture, the inflammatory response, wound healing, and tumor metastasis. The adhesion molecule and/or receptors thus influence many biological activities and processes including cell proliferation, differentiation, cell junction formation, and polarity by targeting cell-surface adhesion to specific ECM proteins and ligands on adjacent cells (Table IX). Multiple families of adhesion molecule/receptors have been identified as the following: (i) the heterodimeric integrin receptors; (ii) the immunoglobulin-like adhesion molecules; (iii) the homophilic cadherin calcium-dependent proteins; (iv) the LEC-CAMs with lectin-like domains; and (v) homing receptors that target lymphocytes to specific lymphoid and nonlymphoid tissues (i.e., CD44) (119). The recent finding that cells can respond to signal transduction from the ECM *via* integrin receptors is highly significant (120). The signal apparently is transduced through the integrins linked to intracellular talin, vinculin, and actinin and transmitted to the actin cytoskeleton. Extensive signalling "cross-talk" can occur between the receptors and other CAMS involving both tyrosine kinases and polyphosphoinositide hydrolysis. It is thought that induction messages of early immediate genes can then be transmitted to the nucleus *via* actin-associated cytoskeletal elements (120).

AFP and the albuminoid superfamily members have been proposed to comprise a system of adhesion molecules for epithelial cells comparable to the immunoglobulin superfamily for lymphoid cells (121). The immunoglobulin superfamily of proteins, which includes immunoglobulins, T-cell receptors, and histocompatibility antigens can accommodate an enormous amount of amino acid sequence variability (see "Immunoregulation and Human Disease" below). The albuminoid superfamily consists of serum albumin, α-albumin, AFP, Vitamin D–binding protein; however, some also include the C700 and B700 melanoma antigens, and kinetensin (89, 122; see "AFP as a Modular/Cassette Protein" above). ALB superfamily members have capabilities or properties that support their candidacy as cell adhesion molecules, namely; (i) transport/carrier function; (ii) enzyme-like activity; (iii) conformational flexibility; (iv) receptor transfer of metabolites; and (v) homophilic

Table IX. Potential Cell Adhesion and Cell-to-Cell Contact Proteins Displaying Human α-Fetoprotein Amino Acid Sequence Identities

Protein name/type	Amino acid sequence identity (%)	Amino acid length (#)	Human AFP domain
C-CAM	30	24	2
Myelin-associated glycoprotein	63	13	2
Osteopontin	75	8	3
E-selectin	75	8	3
E-cadherin	100	5	3
Ph-20 protein	60	10	3
Fasciclin	67	9	3
Muc-18	67	9	3
GAP protein	50	10	3
Catenin	40	25	3

Note. Only sequences showing greater than 30% identity are listed; however, short amino acid lengths are displayed, since triamino acids (RGD) are known adhesive agents.

(dimeric) association or aggregation. It is the dimerization activity of adhesion molecules (i.e., N-CAM, Ng-CAM and L-CAM) mediated by homotypic and heterotypic cell-to-cell adhesion that results in bridging at cell surfaces. ALB superfamily members have long been recognized to associate with each other to form dimers and oligomers, to bind fragments (mini- and microalbumins), and to form highly stable pairs with other protein molecules (82, 123, 124). Thus, AFP and other albuminoid molecules seem well suited to this cell contact function.

Teleologically, it would be important if a major fetal protein were to possess adhesion recognition sequences in its primary structure. Adhesion capabilities could provide a protein with the modulatory properties of cell growth and differentiation, cellular attachment and migration, cytoarchitectural structuring, and morphogenetic movements during both embryogenesis and histogenesis. Examples of such modulation have been reported with the integrin family receptors, beginning at gastrulation and continuing throughout development, involving processes such as neural and myoblast migration and neurite extension on Schwann's cells (119). In addition, some adhesion molecules (i.e., tenascin) are known to consist of both adhesive and antiadhesive domains or signal-recognition sites (125). Thus, it would not be surprising to detect ECM, adhesion, and antiadhesion signal-like amino acid sequences on the AFP molecule. Such potential matches have been detected from the computer-derived data (see below).

HAFP appears to display several adhesive sites of the short variety (3–10 AA) in addition to longer sequence homology to the adhesive molecules themselves. Examination of the primary amino acid sequence structure of HAFP reveals the presence of both RGD (AFP amino acids 252–254) (arginine-glycine-aspartate) and LRE (leucine-arginine-glutamate) adhesion recognition sites (126, 127). The RGD functions as a cell-attachment site in several different extracellular matrix glycoproteins including fibronectin, thrombospondin, von Willebrand's factor, and vitronectin. The LRE triamino sequence (AFP amino acids 194–196) is a crucial determinant for the binding of biliary motoneurons to s-laminin and also serves to inhibit neurite axonal outgrowth at synaptic sites (128).

HAFP, as shown in Tables VIII and IX, displays some sequence homologies with a multitude of ECM and adhesion proteins as detected in Genbank. Examination of the ECM protein identified in Table VIII revealed different matrix-related molecules including plasma-secreted, cell surface, basement membrane, enzyme, and fibril forms. It can be observed that most AA sequence identities were localized on HAFP domain 2 with only two sequences on domain 1. In fact, the majority of protein homologies resided on domain 2. Overall, the amino acid sequence identities ranged from 30% to 47% over stretches of 17 to 28 amino acids in length, respectively. The proteins included collagens, fibrin, and thrombotic molecules related to clot formation/dissolution, platelet attachment, and serine protease activities. It is thus evident that HAFP domain 2 and to a lesser extent, domain 1, may be associated with ECM activities, which could encompass hemostasis, basement membrane architecture, fibril scaffolding and crossbanding, cytoskeletal attachment, and signal transduction.

In comparison with the ECM protein homologies, the cellular adhesion and cell-cell contact proteins show an entirely different pattern of identities (Table IX). It is obvious from Table IX that the adhesion-protein identities were mostly detected in the second half of the HAFP molecule, mostly on domain 3. Only two cellular adhesion sequences were localized to domain 2. In contrast to the ECM proteins, the CAMs showed higher amino acid sequence identities (30%–100%) over shorter amino stretches (5–25 AA). The CAM homologies include proteins implicated in homophilic and heterophilic adhesion (binding), cell attachment and spreading, morphogenetic movements, myelin sheath formation, tissue organization, segment polarity, and regulation of cell proliferation/differentiation. These include proteins which contain Ig-like and lectin-like domains, Ca^{2+}-dependent motifs, cytoskeletal binding segments, and tumor suppressor properties. Since the amino acid sequence lengths are shorter than the ECM sequences, the significance of these observational trends has yet to be ascertained. However, sequences as small as three amino acids (i.e., RGD and LRE) have proven to play crucial roles in the biological activities of the ECMs as discussed above.

Oncogene Transcription and Expression

Transcription Factors, Tumor Suppressors, and Homeodomain Proteins. The oncoproteins are nuclear phosphoprotein products of the proto-oncogenes, which now include *myc, myb, fos, jun, ski, ets, cbl, erb* A, and possibly many others (129). Most oncoproteins have turned out to be transcription factors, which function as molecular switches that sense incoming signals and modulate the transcription of specific genes (130). Although predominantly localized in the nucleus, they are capable of mediating specific transcriptional responses to signals originally generated in the plasma membrane or cytoplasm (131). Some, like *myc,* are present in nearly all cell types, while others like *myb,* are restricted largely to hematopoietic cells (132). Many oncoproteins have functional partners with which they heterodimerize to bind DNA, such as *fos* and *jun, myc* and *max,* etc. Like other proteins, the oncoprotein transcription factors are synthesized in the cytoplasm and transported into the nucleus *via* a nuclear localization signal within their primary structure (70, 130). Protein-protein and protein-DNA interactions of the nuclear oncoproteins are often mediated through helix-loop-helix and leucine zipper motifs (133).

Many oncoproteins have been implicated in the control of normal cell growth and proliferation, cell-cycle control, and apoptosis. In cancer, they may be involved with growth deregulation in mutations that activate the oncogenic potential of cellular proto-oncogenes that lead to the loss of con-

trol of normal cellular proliferation and differentiation. Such activating mutations, as seen in growth-suppressing proteins (p53 and retinoblastoma protein), which bind transcription factors, constitute fundamental steps in the development of human cancers. Although the precise steps in molecular oncogenesis are unknown, mutations can inactivate certain oncoprotein properties such as nuclear localization, transcriptional activation, oligomerization (dimerization), and DNA binding (134). In the case of c-*erb* A, dominant-negative mutations can inhibit the transcriptional regulatory function of the thyroid hormone receptors, resulting in a loss of hormone responsiveness and hormone-induced differentiation (135). This is comparable to the tumor-suppressor genes, where a loss of function can induce the transformation process. In this way, normal hormone-activated nuclear receptors, such as the thyroid and retinoic acid receptors, can function as growth suppressors since their differentiated cellular targets lose their proliferative potential (136).

Among the transcription factors, the discovery of the homeobox gene superfamily that translates the homeodomain proteins remains the hallmark discovery in embryonic development (137, 138). These embryonic induction factors have long been sought, since Hans Speman first speculated their existence in the early 1900s. First discovered in insects, homeobox proteins and their homologs are now known to exist in nematodes, rodents, humans, plants, and yeast (139). Homeoproteins serve to direct and control pattern/positional body development in embryos concerning anterior/posterior, trunk-thorax segmentation, dorsal/ventral axis, body polarity, neural-tube formation, and caudal/gut formation. In fact, birth defects are often homeotic transformations resulting in developmental anomalies in which one part of the body develops in the likeness or dissimilarity of another. Pattern formation in the embryonic germ layers usually involve a network of feedbacks between intrinsic programs of gene expression in developing precursor cells and extrinsic signals exerted by the surrounding embryonic environment (140). The homeotic proteins frequently direct or mediate inductive pathways that partition early axial germ layers into structures or segments with distinct regional identities. These morphogenetic processes are then linked to the terminal differentiation of that particular germ-layer derivative. Examples of the homeodomain proteins would include Pou, antennepedia, crumbs, Wnt, sonic hedgehog, forkhead, and Pax (137).

The potential transcription factors, tumor suppressors, and homeodomain protein sequence identities to HAFP are cataloged in Table X. As shown in the table, amino acid identities for the transcription factors ranged from 29 to 54% over lengths from 13 to 24 AA, largely in HAFP domains 1 and 2. In comparison, the tumor-suppressor (retinoblastoma) protein (141) identities appear to reside only on domain 1 of HAFP. However, a Fat-protein (cadherin-related) site (142) was detected on domain 3 (not shown), demonstrating a 100% amino acid identity but over only a 5–amino acid length. Thirdly, the homeodomain proteins exhibited amino acid identities ranging from 41% to 67% over stretches of 10–27 AA in domains 1 and 2. However, shorter stretches of amino acids were also detected on domain 3 (67%–100%), but their significance is questionable in such short lengths (see below). However, homeodomains are characterized by only four conserved amino acid residues in the C-terminal third domain of all nonyeast homeotic proteins (139). Another 8-AA positions of homeotic proteins are highly conserved, though not invariant, in a stretch of 40 AA N-terminal to the tetra-amino acid signature sequence.

Since these homeotic proteins are present during early embryogenesis, it would seem reasonable that AFP might

Table X. Potential Transcription-Related Factors, Tumor Suppressors, and Homeodomain Proteins Demonstrating Human α-Fetoprotein Amino Acid Sequence Identities

Protein name/type	Amino acid sequence identity (%)	Amino acid length (≠)	Human AFP domain
A. Transcription factors			
c-*erb* A	40	20	1
c-*myc*/*rel*	50	15	1
myb	54	13	2
ras	29	24	2
B. Tumor suppressor proteins			
Rb protein 1	32	25	1
Rb protein 2	50	12	1
Rb protein 3	32	28	1
C. Homeodomain proteins			
Pou domain 1	47	15	1
Antennepedia	64	11	2
Pou domain 2	48	21	2
Xenopus homeobox	42	19	2
C. elegans homeobox	41	27	2
Pou domain 3	67	10	3
Pit Pou	56	10	3

Note. Only sequences showing a least approximately 30% identity and 10-AA length are listed.

display short homeodomain sequences in molecular mimicry of these pattern-regulating proteins. For example, it is known that mutations in the Pax-3 domain result in central nervous system disorders relevant to AFP such as anencephalies and spina bifida, in addition to abnormalities associated with neural crest-derived structures (137). In mammals, Pou domains are expressed during early embryogenesis in many regions of the developing brain including the forebrain and nerve cord. Aside from binding the major groove of DNA, the Pou domain is required for homodimerization and heterodimerization of the Pou proteins. Interestingly, a dimerization domain has been postulated to reside in the third domain of HAFP in a similar fashion to the c-*erb* A nuclear receptors (133). The *Pit-1* gene of the Pou domain controls development of the anterior pituitary, and mutations of this gene display failure of adenohypophysis development. The *Wnt* gene codes for proteins (86%, 7 AA, domain 3) that are expressed in the midbrain-hindbrain border and mutations in this gene result in the absence of these brain regions (143). Finally, crumbs protein (such as found on HAFP 100%, 7 AA, domain 3) mutations have led to severe disorganization and degeneration of ectodermally derived embryonic epithelia (144). Thus, it may be more than a coincidence that human AFP segments share short amino acid sequence homologies with the homeodomain proteins, which are endowed with embryonic body-positional information.

AFP and Programmed Cell Death. Mammalian development is accomplished by a combination of cell proliferation, differentiation, and apoptosis (programmed cell death). Apoptosis routinely occurs during embryogenesis, histogenesis, metamorphosis, endocrine-dependent tissue atrophy, and normal adult tissue turnover (145, 146). Apoptosis can be distinguished from necrosis, a condition that results from pathologic injury, complement-based cellular attack, severe hypoxia, hyperthermia, lytic viral infection, and toxin exposure. Apoptosis is characterized by nuclei condensation and segmentation, DNA degradation, membrane microvilli loss, and cell surface blebbing. Apoptosis occurs normally in the immune system (lymphocytes, thymocytes) induced by antigen-receptor complexing or by glucocorticoid induction (147, 148). Natural killer cells or cytotoxic T cells are also capable of apoptotic induction in target cells. Tumor regression is often mediated through apoptosis as a result of UV, X-irradiation, and chemotherapeutic exposure in cancer cells (149).

Throughout mammalian development, and especially in the early embryo, a competition exists between cell death signals and survival (rescue) signals. For example, gastrulation in the mouse embryo is accomplished by two signals; an apoptotic induction that forms the inner cell mass and a rescue (reversal) signal that allows the survival of columnar cavity-lining cells (130). Although the programmed cell death signal occurs in the inner mass cells, the rescue command is thought to be contained within the extracellular matrix cells in juxtaposition to the ectoderm. It is in this fashion that the embryo forms tubes with a single layer of cells lining the lumens. Cell death helps to create lumens in a variety of embryonic structures, with the dead cells rapidly disappearing by phagocytosis. It has further been shown that the integrin receptors of the ECM play a role in regulating apoptosis during mammary differentiation in the mouse (130).

Growth enhancement by AFP has long been reported in the scientific literature. However, the question of whether AFP contains bona fide growth factor or mitogenic activity is still open to question. The mechanism of the growth-regulatory properties exhibited by AFP in a decade of reports has yet to be elucidated and is largely unstudied. In this regard, an amino acid sequence identity site related to growth hormone (46% over 11 AA) was detected on the first domain of HAFP. A previous report had demonstrated that purified HAFP, together with platelet-derived growth factor, synergistically enhanced the proliferative activity of human medullary breast-carcinoma cell cultures (150). Moreover, AFP was not found to be mitogenic in these studies. It may be germane to this report that HAFP displays an amino acid stretch on domain 1 with identity to the platelet-derived growth factor–α receptor (29% identity, 21-AA length). A more recent study demonstrated that AFP and AFP-receptor antibody blocked the induction of apoptosis in HL-60 leukemia cells in culture (151). The induced cell death by AFP was associated with cellular adherence in microtiter plates. Cell death was assessed by morphology, shrinkage of dying cells, inducibility and reversibility kinetics, and DNA fragmentation patterns. Concomitant studies by others had already shown that cells cultivated on substrates that prevent cell adhesion rapidly progressed into apoptosis (152).

It has now been ascertained that the signal for programmed cell death is mediated by a cell-surface transmembrane protein termed the Fas antigen belonging to the tumor-necrosis factor/nerve-growth factor receptor family (147). Likewise, it has determined that the rescue signal from apoptosis is conferred by the cytosplasmic Bcl-2 protein derived from β-cell leukemias and lymphomas (148). As stated above, a delicate balance exists during mammalian development regarding cell death and rescue (survival) signals for cell growth, proliferation, and differentiation. Hence, it might be feasible to detect remnants of both signal types on an individual growth-regulating protein.

On the basis of Genbank identification, an amino acid sequence resembling a Fas-like peptide stretch is readily detectable on domain 1 of HAFP (39% identity; 23 AA in length). In a similar fashion, slightly downstream on domain 1 of HAFP, a sequence stretch identifying with the tumor necrosis factor receptor type 1 can also be discerned (50% identity, 12 AA in length). Both stretches of amino acids lie in juxtaposition to each other on the first domain of HAFP. One might speculate that dimerization or binding of AFP to these signal proteins could blunt the cell-death signal resulting in enhancement or continuation of cell growth. In con-

trast, a rescue/survival signal represented by a Bcl-2–like amino acid stretch was detected at the opposite end of the molecule on HAFP domain 3 (41%, 17 AA in length). Interestingly, this Bcl-2 site is localized adjacent to a proposed hinge region, which allows rotational flexibility. A conformational change in the tertiary structure of AFP, possibly induced by ligand binding or shock signal, could expose such a site (i.e., Bcl-2), which was normally hidden in a molecular crevice (92). As above, one might speculate that dimerization or binding of AFP to a Bcl-2 protein could block the rescue/survival signal, resulting in the induction of cell death. AFP, being a fetal protein, might be expected to display a FAS-like growth enhancement (domain 1) segment in its normal molecular configuration, while a concealed Bcl 2 interface might surface following a stress-induced conformational change. In this fashion, AFP would normally function to enhance growth while conditions conducive to stress or shock (heat or glucose shock, hypoxia, hyperthermia, excessive ligand exposure) might expose a signal site resulting in cessation of growth or cell death. In this fashion AFP might function in both the up- and down-regulation of growth by employing a binding or dimerizing mechanism to apoptotic mediators. It is unlikely that the inverse is true (AFP directly mediating apoptosis by exposure of signal sites) since normal fetal growth flourishes in the presence of high AFP plasma concentrations.

AFP and Histogenesis. The eye lens in vertebrates and invertebrates is an avascular tissue that allows focus-imaging on the retina (153). The lens grows throughout life, maintaining transparency without significant turnover of its closely packed proteins. The crystallins are water-soluble proteins that form the lenses of vertebrates and cephalopods. In vertebrates, only the 20-kDa β/γ-crystallin proteins are lens specific. The other crystallin classes (α-, β-, δ-, etc.) of proteins are expressed in the lens as well as other tissues of the body. α-Crystallin has been reported to act as a chaperone under conditions of oxidative stress (154). The β- and γ-crystallins are closely packed in layers by protein-to-protein dimerization of the amino- and carboxy-terminal domains in packed aggregate layers (155). Due to aging, glucose glycation, diabetes, and autoimmune disease, fogging of the lens and cataract formation result in blurred vision. Glycation of rat crystallin can be inhibited by aspirin. Anticrystallin autoantibodies in humans are largely directed against the lens-specific proteins (156, 157) and crystallin fragments (9 kDa) appear to increase in quantity in human lenses during aging.

All three domains of HAFP express amino acid sequence-identity sites with the delta crystallin proteins. A large sequence length was detected on domain 1 with a 24% identity over 85 amino acids. Within the confines of this domain 1 region, a human lens membrane protein was also detected displaying a 28% identity over 37 AA. Coincidently, as previously discussed, this domain of HAFP also contains an identity site for the retinoblastoma protein (see Table X), which is known to be required for lens fiber-cell differentiation (158). On HAFP domain 3, another δ-crystallin amino acid sequence stretch was found exhibiting a 36% identity over 22 AA. Interestingly, an identity site for a rhodospin/opsin vision protein was also detected (41% identity, 29 AA) on the third domain of HAFP. Finally, two identity sites in juxtaposition were found at the carboxyl terminus of HAFP. The first site displayed a 63% identity over 8 AA, while the more terminal site exhibited 47% over 15 AA.

It may be no mere coincidence that AFP constitutes a major determinant in the development and differentiation of the sensory organs as published works would indicate. Studies of the role of AFP in eye development have indicated that antibodies to AFP are capable of disrupting eye histogenesis in chick retinae cultures (159). Seventeen-day retina cultures treated with antibodies to chicken AFP in organ culture for 3 days failed to form both the plexiform layers and photoreceptor cell buds. Further studies in 14-day-old rat embryos have implicated AFP in the development of olfactory placode-derived neurons in the nasal pit of the brain (160). A rat olfactory marker protein has also been localized on HAFP domain 2 displaying 48% identity over 21 AA in length.

Ligand Binding and Carrier/Transport

Ligand Binding Regions on AFP. There is now overwhelming evidence that AFP is an embryonic and fetal carrier/transport molecule for a multitude of ligands including fatty acids, bilirubin, heavy metals, steroids, retinoids, drugs, dyes, and antibiotics (161–163). However, the precise binding location on the AFP molecule is known only for a few ligands. For example, a major fatty acid–binding site for long-chain fatty acids has been documented to lie between amino acid residues 210 and 227 on HAFP domain 2 (164). Lysine residue 223 appears to be the essential amino acid for the fatty acid binding. Studies employing Scatchard binding/saturation analysis had previously demonstrated that at least three possible ALB binding sites ($K_a = 10^{-7}$ M, $n = 3$) exist for the polyunsaturated fatty acids (i.e., arachidonic and decosohexanoic acids) (165, 166). If AFP is similar to ALB in this regard, then it is most probable that one fatty acid–binding site exists on each of the three domains, and such may indeed be the case (167). The remaining two fatty acid–binding locations can only be speculated on the basis of Genbank-derived amino acid comparisons to fatty acid–related proteins. One such example of a potential HAFP domain 1 site residing at residues 36–69 shows an amino acid homology to fatty acid synthetase (28% identity, 32 AA). Another possible site on domain 1 could reside at AA 170–203, wherein lies another fatty acid protein–like stretch (32% identity, 19 AA in length, together with a kainic acid–binding protein). On domain 2, a fatty acid denaturase homolog site was identified on AA 223–237 overlapping the confirmed fatty acid–binding site at residues 210–227 (see above). The third AFP binding site resides on domain 3 and apparently overlaps the

estrogen-binding site on rat AFP according to previous competitive-binding reports (75, 168).

The estrogen-binding interface on rodent AFP has been determined to occupy a region between amino acid residues 423 and 444 forming an α-helix segment lying adjacent to a β-sheet/turn structure extending from residues 445 and 480 (96, 97). The former site represents a major hydrophobic binding pocket on HAFP that does not bind estrogenic steroids, in contrast to the rodents (169). In humans, this region (amino acids 423–480) displays overlapping binding sites for fatty acids, DES, protease inhibitors/substrates, retinoids, warfarin, coumarin, phenylbutazone, pyrazolic drugs, and anthranilic acid (161, 162). Previous competitive-binding studies employing rat AFP determined that estrogen was bound on RAFP domain 3 together with retinoids and fatty acids.

Employing competitive inhibition-binding analysis, it was determined that bilirubin did not compete with fatty acids for the same binding sites on HAFP, both sites were totally distinct from the retinoid-binding region (163, 171, 172). Earlier studies had already ascertained that HAFP possessed two separate binding sites for bilirubin while ALB has three sites for this heme pigment (173). The former study concluded that HAFP bound both fatty acid and bilirubin noncompetitively on domain 1 and domain 2, while steroids and retinoids were bound on domain 3 (173). Regarding bilirubin-binding on HAFP, the two sites appear to reside on either side of the single tryptophan residue on HAFP as determined by spectral and competitive-binding analyses. With this information, one can speculate concerning the location of the two bilirubin binding regions in light of Genbank amino acid identities. The domain 1 site might well be positioned in the amino-terminal direction to the tryptophan (present at amino acid 162) between amino acids 137 and 169. At this position, HAFP AA identities were found with pigment-associated proteins (β-globin, 40%, 20 AA in length; chloroplast protein, 35%, 29 AA in length). In comparison, the domain 2 site might be located in the carboxy-terminal direction to the tryptophan residue and to the known fatty acid–binding site. The second bilirubin site can be predicted to lie between amino acids 239 and 276, where pigment-related protein amino acid identities were also detected (heme oxygenase, 33%, 12-AA length; cytochrome-*c* oxidase, 82%, 11-AA length; a chloroplast protein, 46%, 13-AA length; and phycoerythrin, 58% identity, 19-AA length). It may be of interest that a benzodiazepine receptor homology site has also been identified at this same region (amino acid identity 38%, 24-AA length) that includes a serotonin receptor region at amino acids 242–269 (amino acid identity 42%, 12-AA length). Both of these latter compounds have nitrogen-ring aromatic hydrocarbons that mimic those of bilirubin. In contrast, ALB has these latter binding sites on domain 3 (174).

The binding sites of heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, etc.) on HAFP have not been precisely determined; however, multiple binding sites have been reported (175, 176), as have AFP purification methods using metal-chelated column chromatography (177). An *in vivo* study of rodent AFP exposure to high concentrations of heavy metals has also been reported (178). Early studies of ALB binding to heavy metals frequently implicated the amino-terminal domain (175); AFP could well be similar. Most investigators support the involvement of histidine residues in the binding of Cu^{2+} and Zn^{2+} ions, probably at the imidazole group (175, 176). Cysteine residues have also been implicated with Zn^{2+} in the binding to DNA (179); HAFP has only two free cysteines, located at amino acid residues 18 and 67. These residues of free cysteines occur in the first domain of HAFP in keeping with the ALB metal-binding reports. It may be no mere coincidence that HAFP sequence identities with Cu/Zn-superoxide dismutase occur in amino acids 1–69 (31%, 32-AA length; 60%, 10-AA length). A Cu^{2+}-transporting ATPase identity stretch (26%, 19-AA length) was also found between amino acids 137 and 169, wherein lies an Arg-His-Pro sequence in domain 1. On the second domain of HAFP, a histidine-rich region is found at amino acid residues 224–230 that contains four histidines. This site could easily qualify as a major Cu^{2+}- and/or Zn^{2+}-binding region since it overlaps a Cu^{2+}-transporting ATP identity site (33%; 21-AA length) located there. From these data, one could speculate that heavy-metal binding can largely be assigned to HAFP domains 1 and 2.

AFP as an Antioxidant. The importance of free radicals in the biomedical sciences is becoming increasingly evident since the realization that antioxidant protection may have therapeutic applications (180). Although the enzymes that scavenge radicals, such as superoxide dismutase, have garnered much of the recent attention, the antioxidants that prevent free radical formation (catalase, glutathione peroxidase) are likewise important in inhibiting the cascade of free-radical generation. Molecules such as albumin, transferrin, lactoferrin, and caeruloplasmin have long been known to function as inhibitors of free-radical formation in plasma *via* the sequestration of ions of transition metals such as copper and iron (181). For example, copper ions are able to accelerate damaging free-radical reactions, while albumin is able to markedly inhibit copper-stimulated peroxidation and hemolysis of erythrocyte membranes as well as copper-dependent lipid-peroxidation systems (182). Once the albumin binds the copper ions, H_2O_2 is still accessible to the bound transition metal so as to cause the formation of hydroxyl radicals; hence, damage is focused upon the albumin molecule. The damaged (denatured) albumin may be proteolytically degraded, but is quickly replaced. Albumin is also capable of preventing the inactivation of α_1-antiprotease by binding hypochlorous acid, generated from the neutrophil myeloperoxidase enzyme, which uses H_2O_2 to oxidize chloride ions to form the acid (183). Finally, albumin is reported to scavenge peroxy radicals and decrease lipoxygenase activity and to bind free fatty acids to protect them from peroxidation (184).

Outside the plasma, albumin concentrations are much

lower in cerebrospinal fluid, aqueous humor, synovial fluids, and bronchial lining fluids (185). Vascular permeability is increased at sites of tissue damage by oxygen-derived products secreted by neutrophils, which leads to increased vascular permeability. In the lungs this produces alveolar-capillary leaks, while, in synovial membranes, increased permeability induces inflammation in joint disease. Exposure to prolonged high O_2 (hyperoxia and respiratory distress syndrome) results in infiltration of neutrophils that generate hydroxyl radicals, which inactivate α-antiproteases. Interestingly AFP is elevated in cases of perinatal respiratory distress syndrome (186). However, increased vascular permeability serves to elevate the extracellular fluid content of the scavenging plasma proteins, which in turn helps to prevent further excessive damage by the oxidants. As discussed below, it is conceivable that AFP might likewise serve as an antioxidant on the basis of amino acid domain and motif structure. Similar to albumin, AFP is capable of binding and sequestering the ions of transition metals such as copper and zinc as well as non-esterified long-chain fatty acids (i.e., arachidonic and decosohexanoic acid) (180, 181, 185, 187). As described above, glutathione peroxidase (GTP) functions to prevent free-radical formation, while albumin, and probably AFP, function as inhibitors of such reactions. The human plasma glutathione peroxidase (containing selenocysteine) serves as a major enzyme in reducing lipid and phospholipid hydroperoxide and H_2O_2 in plasma (188), and a gastrointestinal enzyme form has also been described (189). As shown in Table XI, HAFP displays an overall amino acid sequence identity/similarity to a rat lung glutathione peroxidase (GTP) that is associated with the development of tolerance of the animals to hyperoxia (190). This enzyme was found localized in both rat lung and liver. It is evident from Table XI that glutathione peroxidase ranked first in sequence homology among five proteins (other than albumin) selected from Genbank. The rat GTP displayed 24% identity and 42% similarity to HAFP, while BRCA-1, a human breast-cancer tumor suppressor protein, surprisingly ranked second in this random survey with a 21% identity and a 44% similarity. The latter amino acid similarity of BRCA-1 even exceeded the GTP homology. Unexpectedly, the albuminoid gene family member vitamin D–binding protein was positioned midway in the rankings, trailing leucine peptidase and preceding the retinoblastoma protein. Nevertheless, these data suggest an AFP amino acid relationship to GTP, clearly not a gene family member. This relationship would imply that HAFP might be involved with free-radical chemistry, possibly by molecular mimicry in addition to heavy-metal sequestration.

HAFP has been reported to bind both Cu^{2+} and Zn^{2+} ions in a carrier/transport fashion. Possible candidate sites for such binding can be predicted from data extrapolated from Genbank amino acid sequence homologies/identities. For example, HAFP displays a Cu, Zn-superoxide dismutase homology on domain 1 (31% identity over 32 amino acids) and a copper-transporting P-type ATPase on domains 1 and 2 (26% identity over 19 amino acids; and 44% identity over 18 amino acids, respectively). Thus, it is tempting to speculate that HAFP might bind copper and zinc on domains 1 and 2 as discussed above. Domain 1 is a strong candidate since two free cysteine residues are located therein, while domain 2 contains histidine-rich regions (see above).

The myeloperoxidases (MPO) encompass a family of homologous mammalian peroxidases that include thyroid peroxidase, eosinophil peroxidase, and lactoperoxidase (191). These enzymes employ a heme prosthetic group bracketed by histidine and arginine residues typical of lactotransferrin (192). It is also of interest that thyroid peroxi-

Table XI. Human α-Fetoprotein (HAFP) Sequences Compared With Various Human Proteins

A. The entire amino acid sequence (590 AA) of HAFP compared with other human proteins

Protein	Amino acid stretch	Identity (%)	Similarity (%)
Human albumin	1–590	39.96	59.34
Glutathione peroxidase	1–513	24.19	42.28
BRCA-1	349–1153	20.87	43.83
Retinoblastoma protein	1–3000	19.43	42.27
Vitamin D (Gc)	1–536	19.00	38.00
Leucine aminopeptidase	1–8305	16.99	40.43

B. The three domains of HAFP compared with other human proteins

Protein name/type	Domain 1 identity/similarity (%)	Domain 2 identity/similarity (%)	Domain 3 identity/similarity (%)
Albumin	32/44	41/45	47/41
Glutathione peroxidase	24/49	21/47	28/48
Retinoblastoma protein	22/55	15/48	19/44
Vitamin D (Gc)	20/49	22/46	24/45*
Myeloperoxidase	18/44	17/36	16/33

* Truncated at 103 AA on carboxy terminus.

dase is a human autoantigen involved in the tissue destruction observed in thyroiditis (see "AFP and Programmed Cell Death" above). AFP may be similar to albumin in preventing the inactivation of α_1-antiprotease by binding neutrophil-derived myeloperoxidase generated as a response to hypochlorous acid output (185). This suggestion is in keeping with sequences generated from Genbank. In Table XIB, it can be observed that the entire domain 1 of HAFP displays 24% amino acid identity and 49% similarity to rat glutathione peroxidase and 18% and 44%, respectively with human myeloperoxidase. Mouse albumin registered a distant third in the ranking. In domain 2, a similar but slightly lowered percentage was evident in the peroxidase, while mouse ALB was slightly increased. However, in HAFP domain 3, glutathione peroxidase increased to 24% sequence identity, while myeloperoxidase remained constant. It is in domain 3 that mouse AFP increases to 33% identity and 52% similarity. Thus, the peroxidases vary in their proposed identity/similarity to HAFP in a domain-dependent fashion.

To what advantage would a fetal protein similarity to the peroxidases have during development? It has recently been ascertained that the myeloperoxidase (MPO) gene is tightly regulated in a tissue and developmental-stage manner during organogenesis (193). MPO-DNA is highly methylated, restricting transcription to only the late myeloblastic and promyelocytic stages of myeloid (bone marrow) differentiation. In cells developmentally too immature to transcribe MPO, AFP might be useful in the liver and bone marrow as a molecular mimic of MPO with the capability of sequestering copper and zinc ions and possibly hypochlorous acid.

Interactions of AFP with Hormones and Growth Factors. Reports of AFP interactions with a variety of hormones can readily be found in the literature. Such findings include hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), retinoids, thyroid hormones, and others. More recently, AFP interactions with growth factors have also been recorded. Studies in the mid-1970s indicated that AFP was indeed subject to hormonal, nutritional, and hematological regulation (194). These results indicated that prednisolone, adrenaline, and thyroxine depressed AFP levels through a selective blockade of its synthesis. In the case of thyroxine, depression of AFP synthesis has been confirmed both in cell culture studies (195) and in clinical follow-up of hypothyroid newborns (196). In contrast, estradiol (E2) and estrone treatment induced the synthesis of AFP in the livers of adult rodents (197, 198). Concomitant *in vitro* studies further showed that AFP was able to inhibit the formation of water-soluble metabolites of estrone and estradiol by incubation with microsomes from rat livers in the presence of NADPH (199).

In immature female rats, the percentage of unbound E2 is low until 21 days of age, when the proportion of free hormone rapidly increases. The rise in unbound E2 is accompanied with a known fall in FSH levels coupled with a concomitant disappearance of AFP from the circulation (200, 201). The administration of AFP extracts has resulted in a significant rise in plasma FSH and a lower proportion of unbound E2 (200). The maturation of the inhibitory feedback action of estrogen on FSH secretion in the immature female rat has been reported using moxestrol, an estrogenic steroid not bound by AFP (201, 202). It was concluded from these studies that (i) the increase in sensitivity to the feedback action of estradiol from 20 to 28 days reflects the disappearance of AFP from rodent blood, and that (ii) estradiol is the sole ovarian factor regulating FSH secretion in rats up to 20 days of age. Subsequent studies confirmed that the decline in serum AFP was strongly linked to the progressive increase in tissue-to-serum ratios of estradiol during the first 5 weeks of life (203) and that during these weeks an LH-surge developed in a progressive stage-wise fashion (204). More recent evidence, employing free E2 and nuclear-bound E2 measurements, confirmed that AFP injections extended the period of low serum unbound E2, depressed levels of nuclear-bound E2, and delayed the onset of puberty (205). In lieu of such studies, it may be of interest that an FSH β subunit sequence identity (28%, 29 AA) site has been localized to the third domain of HAFP situated in juxtaposition to the recently discovered estrogen binding pocket homologous to the rodent AFP site (96).

It has long been known that AFP was capable of suppressing estrogen action on target tissues *in vivo*. Employing mitotic indices, it was first demonstrated that AFP administered together with E2 significantly suppressed E2-induced vaginal epithelial cell proliferation (206). It was further observed that hepatoma-bearing rats (AFP-secretors) remained in diestrus, and showed decreased uterine wet weights, depressed serum estrogen levels, and appearance of castration cells in the anterior pituitary (207). When E2-sensitive mammary tumor growth was studied in AFP-secreting hepatoma-bearing rats, mammary tumor growth regression was observed (208). Similar results were reported when the E2-sensitive mammary tumors were implanted into AFP-secreting newborn mice. The addition of purified AFP fractions into newborn rats bearing E2-sensitive pituitary tumor cells also prevented oncogenic growth (209, 210). These authors concluded that AFP was a specific inhibitor of the multiplication of cells that were E2 sensitive for growth. Because of such reports, the author undertook a series of studies to investigate and hopefully elucidate the E2-sensitive growth inhibitory properties of AFP *in vivo* (16–18, 211–213). These studies concluded that rodent and human AFP contained a concealed E2-sensitive growth regulatory site that emerged following exposure to high estrogen concentrations (17). Such a mechanism might account for the prevention of fetal hyperestrinism in the face of high maternal estrogen levels during pregnancy as first purposed by Vannier and Raynaud (214) and more recently addressed by Nunez *et al.* (215, 216). It was this hidden E2-sensitive growth regulatory site on HAFP that was re-

cently produced as a free peptide (92) and is described above in "AFP as a Modular/Cassette Protein."

It has also been reported that AFP regulates *in vivo* the postnatal stages of sexual maturation of the rat ovary. Regulation by injected AFP was evidenced in these studies by (i) decreased numbers of maturing follicles, (ii) corpora lutea number, (iii) drop in ovarian weight, (iv) decreased progesterone levels, and (v) blocked estrus cycles (217). A similar physiological effect could be produced in the postnatal mouse ovary by intracranial injections of rabbit anti-mouse AFP immunoglobulins during the first week of life (218). Histological examination of these mice at Day 60 revealed the presence of polyfollicular ovaries lacking in corpora lutea formation (219). These results appeared to mimic steroidal androgenization of female mice during the perinatal period.

The synergistic action of HAFP with various growth factors has also been reported in porcine ovarian granulosa cell cultures. It was initially found that physiological levels of HAFP could enhance the mitogenic activity of epidermal growth factor (EGF) and transforming growth factor–α (TGFα), suggesting that AFP might serve to modulate growth factor–mediated proliferation during development and neoplasia (14, 15). Further studies on these porcine cell cultures revealed that AFP in the presence of EGF plus insulin-like growth factor–AI (IGF-AI) and platelet-derived growth factor (PDGF) also produced the growth-enhancing effect (15). It may be of interest that a PDGF receptor-like sequence has been detected on the first domain of HAFP (see "AFP and Programmed Cell Death" above). Subsequent use of the granulosal cell cultures seemed to indicate that, while HAFP was capable of growth factor–enhanced growth of these cells *in vitro,* HAFP also inhibited the steroidogenic function of E2 production (220). It was later demonstrated that HAFP actually inhibited the FSH-stimulated E2 production in the porcine granulosa cells (221). These authors suggested that AFP could be inhibiting differentiated functions such as aromatase enzyme activity while enhancing cell proliferation. One might speculate that the FSH-like sequence described above may have relevance to this observation.

Immunoregulation and Human Disease

AFP and the Immunoglobulin Superfamily. *The major histocompatibility proteins.* The major histocompatibility complex (MHC) genes encode three major sets of molecules; the class I, II, and III proteins (222, 223). Class I and II molecules are involved in immunological recognition with the MHC, while class III molecules are concerned with complement cleavage in the inflammatory cascade response. The class I molecules comprise a single transmembrane glycoprotein heavy chain associated with β_2-microglobulin. HAFP showed a class I identity site on the second domain (Table XII). This amino acid sequence stretch was 23 AA long with an AA identity constituting 35%. Class II proteins consist of two noncovalently associated peptides that transverse the plasma membrane at their COOH terminus. HAFP displayed one such amino acid identity on domain 1. The site demonstrated a 25% identity stretch over 25 AA. Finally, the class III molecules (complement-associated) displayed an amino acid identity site on HAFP within domain 2. The amino acid identity site was 62% over an amino acid length of 13 residues. It is tempting to speculate that HAFP could be involved in both MHC and complement interactions. However, it is germane to this discussion that rodent AFP has been reported to selectively inhibit I-region–associated proliferative responses *in vitro* (224). Fetal-derived AFP was shown to markedly suppress proliferation responses against MHC class I determinants mediated largely by Ly1+ but not Ly2+ cells. Thus, AFP was shown to inhibit the recognition and proliferation of T lymphocytes responding in mixed leukocyte culture against histocompatibility-associated alloantigens. In a subsequent publication, another group of inves-

Table XII. Potential Proteins of the Immunoglobulin Superfamily Demonstrating Human α-Fetoprotein Amino Acid Sequence Identities

Protein class	Amino acid identity (%)	Amino acid length (#)	α-Fetoprotein domain
A. Major histocompatibility proteins			
Class I	35	23	2
Class II	25	25	1
Class III	62	13	2
B. The T-cell receptor proteins			
V β-chain	37	19	1
V β-chain	58	19	2
β-chain	38	24	2
δ chain	40	15	3
C. Immunoglobulin heavy-chain proteins & receptors			
IgG receptor (Fc-γ)	30	30	1
Ig heavy chain	30	20	2
Ig (V) heavy chain	32	31	3
IgG receptor	50	12	3

Note. Only sequences showing greater than 25% identity and 10-AA length are included.

tigators demonstrated that murine AFP acted *in vitro* to inhibit macrophage expression of cell-surface Ia antigens, the class II glycoproteins of the major histocompatibility gene complex (225). AFP derived from neonates, amniotic fluid, and hepatoma cells inhibited by 50% or more the expression of Ia proteins in a dose-dependent fashion. AFP, however, was not found to inhibit the direct interaction of lymphokine with the macrophages. These latter two reports lend credence to the supposition that the MHC protein identity sites detected on HAFP might have relevance concerning its immunoregulatory properties.

The t-cell receptors. The MHC controls antigen presentation *via* a three-way interaction involving the T-cell receptor, MHC molecules, and processed antigens. The T-cell receptors fall into two main types, the T-cell receptor–1 (TCR1) and the T-cell receptors–2 (TCR2), both composed of heterodimer combinations of α-, β-, γ-, or δ-chains, constituting constant and/or variable regions (226). The TCR2, for example, is a 90-kDa heterodimer consisting of a 45-kDa α-chain peptide that is disulfide-bridged to a 40-kDa β-chain peptide. Both chains traverse the cell membrane and terminate with short intracytoplasmic tails. The processed antigen appears to bind both a portion of the TCR2 and a portion of the MHC molecule at the surface of the antigen presenting cell. The α- and β-chains each appear to recognize antigens together with the MHC-encoded molecules on the surface of cells. Both helper T cells and cytotoxic T cells express their TCRs in a molecular complex involving three other linked-polypeptide chains termed the CD3. These three chains are noncovalently linked with each other and with the α, β-chains of the TCR. Each chain is a transmembrane peptide and approximately one-third of the chain is intracytoplasmic. Both the TCRs and the CD3 molecules are members of the immunoglobulin supergene family.

Both rodent and human AFP have long been implicated in regulation of immune responses of the humoral and cell-mediated types (227, 228). Thus, it was not surprising that HAFP amino acid identity segments involving TCRs were detected on all three domains (Table XII). HAFP domain 1 bears a β-chain TCR site that displayed a 19-AA stretch bearing a 37% identity, while the domain 2 site possessed a 19-AA sequence with a 58% identity. HAFP domain 2 revealed another site over a 24-AA length with a 58% identity. Finally, the third domain of HAFP demonstrated the presence of a potential site composed of shorter AA lengths representing the δ-chains of the TCR, which displayed a 40% identity over a 15-AA length. One might speculate that some of the reports detailing immunoregulatory functions of AFP may somehow be related to the detection of potential TCR identity sites on the various domains of HAFP.

Immunoglobulin heavy chains. The immunoglobulins (Igs), or antibodies, are a group of lymphoid-derived glycoproteins present in the serum and tissue fluids of all mammals (223). They are produced in large amounts by plasma cells, which have developed from precursor β lymphocytes. Such lymphocytes carry membrane-bound immunoglobulin with the antigen specifities of the plasma cell–secreted Igs (Table XII). These Igs are bound to lymphocytes through receptors present on the cell surface that bind various Ig classes (i.e., the IgG β-Fc γ receptor). Data from the Genbank have detected potential homology sequences largely with Ig heavy chains and with the IgG receptor itself. HAFP domain 1 appear to be devoid of any Ig heavy-chain amino acid stretches; however, such sequences were localized on domain 2 and one on domain 3. The homology on domain 2 was 30% identical with an amino acid length of 11. The Ig heavy-chain sequence on HAFP domain 3 was localized at the carboxyl terminus of the molecule, representing an identity of 32% over 31 AA in length. Finally, two IgG receptor identity sites were detected on HAFP, one each on domains 1 and 3. The IgG receptor identity site on domain 1 displayed a 30% identity over 30 AA, which correspond to a β-Fc γ sequence. The IgG receptor sequence located on the amino-terminal side of domain 3 showed a 50% identity over 12 AA in length. It is conceivable that some of the observations in the literature concerning AFP-binding proteins may be related to heavy-chain Ig binding to AFP (see the following section and "Molecular Forms of AFP" above).

The Autoimmune Disease Connection. AFP has been reported to display immunoregulatory functions in a variety of experimental systems, although the overall findings remain controversial and their interpretations are still being debated (Table XIII). HAFP serum levels in pregnant and nonpregnant adults have also been implicated in a number of clinical autoimmunity reports and controlled case studies, which in turn have prompted further laboratory experimentation. Many of the clinical observations have involved autoimmune disease remission in patients during the latter half of pregnancies when AFP serum concentrations have peaked. The highest frequency of reports has emanated concerning patients with putative autoimmune disorders such as rheumatoid arthritis, myasthenia gravis, multiple sclerosis, and systemic lupus erythematosis. The development of experimental myasthenia gravis in rabbits and rats has also been reported to be prevented by the administration of HAFP (228, 237). These same investigators reported that HAFP was capable of suppressing experimental allergic encephalomyelitis in guinea pigs (238), although such findings were not always confirmed by others (239).

Among the autoimmune diseases observed during pregnancy, systemic lupus erythematosis (SLE) presents an interesting case study. In a large controlled study at a Lupus Pregnancy Center, AFP maternal serum (MS) levels were found higher in lupus than in control pregnancies without the presence of birth defects (229). The increased MS-AFP was attributed to an underlying pathology of placental permeability caused by SLE vasculitis. Any disruption of the maternal-fetal barrier (such as antibody-induced inflammation, premature membrane rupture, placenta previa) will significantly increase MS-AFP levels (186). Other sequelae

Table XIII. Association of α-Fetoprotein with Various Autoimmune Disorders and Its Reported Activity and/or Effect in Nonpregnant and Pregnant Adults

Autoimmune disorder	Activity and/or effect	Reference
Autoimmune hemolytic anemia	AFP inhibits ABO isoagglutination	72
Systemic lupus erythematosis (human)	Increased maternal AFP serum levels	229
Experimental murine lupus	AFP suppressed hypergammaglobulinemia	230
	AFP slowed degradation of Thy-1,2 antigens	
Clinical myasthenia gravis	Temporary remission during/after pregnancy	231
Clinical research myasthenia gravis	AFP binds MG antibodies	232
	AFP reduced muscle weakness and nerve transmission	
Experimental murine myasthenia gravis	decrements	233
Autoimmune-induced arthritis	AFP suppressed disease induction	234
	AFP inhibited onset of disease and binding of antimyelin	
Experimental rabbit allergic encephalomyelitis	antibodies	235
Experimental rat allergic encephalomyelitis	AFP inhibits complement lysis of oligodendrocytes	236

associated with the lupus pregnancies were abnormal AFP levels attributed to prednisone dose, preterm delivery, presence of anticardiolipin antibodies, arthritic joints, and hemolytic anemia (230). It may be of interest that NZB mice, genetically reared to express an SLE phenotype, characteristically display severe forms of hemolytic anemia. Treatment of young NZB mice with AFP, at serum levels of 60–200 μg/ml, significantly reduced the titers of IgG_1, IgG_2, and IgA anti–erythrocyte antibodies. AFP treatment further reduced the hypergammaglobulinemia and slowed the decrement of splenic thy-1,2 antigens seen in nontreated NZB mice (230). However, AFP exposure was not successful in decreasing the degree of lymphoid infiltrates of parenchymal cells nor in preventing the incidence of lymphoma in these mice. There was no change in the median survival nor renal disease of the NZB/W mice at any age; however, the mice did exhibit lower DNA-binding (anti-DNA antibodies) activity in their sera. Although the reasons for these immunoglobulin effects have not been clarified, it may be related to the binding of AFP to autoantibodies directed against the RBC surface antigens (see "Molecular Forms of AFP" above). However, a large portion of HAFP domain 1 has been found to display sequence homology (see below) with the lupus SM-B SLE antigen found in the Genebank (27% identify over 70 AA). The significance of this sequence homology has not yet been determined but may provide the rationale to further explore AFP binding to SLE antibodies (Table III) or other autoantibodies during pregnancy. A comparable situation is already known in pregnant women afflicted with myasthenia gravis as discussed in the next section.

An observation was noted in an Israel treatment center that third trimester and perinatal pregnant women suffering from myasthenia gravis (MG) experienced temporary remissions (231). The possibility was raised that perhaps specific proteins produced during pregnancy, such as AFP, were the participating agents in this clinically observed remission. Since inhibition of MG autoantibodies at the myoneural junction caused amelioration in experimental animal models, it was reasoned that AFP may be competing or binding with the MG antibodies at the acetylcholine (Ach) receptor site. Indeed, inhibition was the case as shown by *in vitro* competitive binding studies employing torpedo electric eel Ach receptors, MG-patients' serum antibodies, and highly enriched HAFP preparations (232). The authors concluded that AFP was binding the Ach antibodies prior to receptor attachment, which could serve to explain the temporary remissions recorded during and immediately following pregnancy. As mentioned above, AFP binding to IgG and IgM has been previously reported (see "Molecular Forms of AFP" and "Immunoglobulin Heavy Chains" above). More recent studies of the passive transfer of MG antibodies in mice have confirmed the ameliorating effects of AFP administered before and after passive transfer in reducing muscle weakness and electomyographic decrements (240). Mice in this study were protected against the onset of the disease and subsequent death which usually followed. The authors reported obtaining evidence for a significant interaction between purified murine AFP and the plasma IgG preparation from MG patients. This is not surprising in light of the Ig heavy-chain amino acid matching to AFP described above.

Elevated maternal serum AFP has also been reported in pregnant women with other autoimmune diseases. As with SLE, the elevated levels have been explained on the basis of increased placental permeability (leakage) to AFP induced by arthritic vasculitis. Using an experimental animal model of antigen-induced arthritis in transgenic mice producing HAFP, Japanese investigators have demonstrated that the continued presence of intrinsically produced serum AFP (20 μg/ml) ameliorates disease development (234). In further studies with mice immunized to methylated bovine serum albumin in adjuvant, transgenic mice displayed arthritis in only 21% of the treated group, compared with 56% in control mice. The AFP serum levels corresponded to maternal AFP concentrations during murine pregnancy but were 500 times higher than adult mouse serum levels.

Concerning experimental allergic encephalomyelitis (EAE) in rabbits, animals were treated with daily injections of 50 μg of HAFP following the onset of neurological signs

(235). Treatment with HAFP significantly improved the clinical scores of the affected rabbits and inhibited the binding of anti–myelin basic protein (MBP) antibodies to purified MBP *in vitro* by 60%. In a comparable study, rabbit antiserum to galactocerebroside (anti-GalC) produces a titer-dependent complement lysis of cultured bovine and rat oligodendrocytes (236). With the concomitant addition of HAFP-enriched fractions, the complement-dependent oligodendrocyte lysis was suppressed from 76% in controls to 31% in the AFP-treated group. Similar findings were obtained when HAFP was directly mixed with anti-GalC antibodies in a competitive radioimmunoassay. The results suggested that the AFP activity may have resulted from binding of the fetal protein to the Fc portion of the IgG antibody involved, interfering with complement binding and the subsequent lysis. HAFP displays amino acid segments on domain 1 and 3 with sequence identities to the IgG receptor Fc portion (30% identity, 30-AA length; 50% identity, 12-AA length). These latter data, together with the Ach receptor binding studies described above, suggest that the antibody-binding effect is not autoimmune disease specific. In each case, the clinical antibody titers were only slightly lowered during AFP treatment and failed to demonstrate a consistent correlation between anti-autoantibody titers and clinical signs of the disease.

AFP has been further shown to inhibit both the humoral and cellular immune responses to EAE and MG in rats and in rabbits (186, 229–232, 234, 235, 238, 239). Although AFP appears to bind to the Fc portion of the IgG autoantibody involved, the exact mechanism has yet to be elucidated. As observed above, in the pregnant state, AFP was effective in improving the clinical health of the research animals, even after the appearance of clinical signs. The interaction of AFP with antibodies to autoimmune antigens may be somehow related to the proposed role of AFP in regulating autoreactive lymphocyte function in the thymus and bone marrow of the fetus and newborn (227). The advent and use of recombinant AFP has demonstrated that AFP-mediated immunomodulation is an activity intrinsic to the molecule itself and is not attributable to either copurifying and/or bound agents or to posttranslational modification events (233). These findings serve to buttress the recent report that HAFP was found to downregulate phorbol ester–induced cytokine production and gene expression in human monocytic cells by a prostaglandin (PGE_2)-dependent mechanism possibly linked to signal transduction pathways (241).

AFP and Disease-Associated Proteins. As mentioned in the section on AFP and autoimmune diseases, a rather long amino acid lupus-like sequence stretch was computer-detected on the first domain of HAFP. It involved a stretch of 70 AA that displayed 27% identity with the mouse Sm-β lupus protein, which is an autoantigen composed of a small nuclear ribonucleoprotein (242). As discussed in that section, a relationship of AFP serum levels with lupus has been reported (229). Again on HAFP domain 1, an amino acid identity sequence with a mouse polycystic kidney disease associated protein was detected. This stretch extended over 12 AA and displayed a 50% identity with AFP. Interestingly, a relationship has been known to exist between the high risk of neural tube defects in diabetic pregnancies (i.e., insulin-dependent diabetes [IDD]) and serum maternal serum (MS) AFP levels (243). On the average, MS-AFP levels in IDD pregnancies are lower than in the general population, and a correction factor is needed to adjust the level for clinical comparison. Diabetic uremia and nephropathologic events are known to accompany polycystic kidney disease in both pregnant and nonpregnant patients (244, 245). In addition, AFP serum and amniotic levels are found elevated in cases of congenital nephrotic syndrome (246). Pathologically, the increased AFP levels are accompanied by absence of foot processes and increased mesangial matrix of the glomeruli.

An AFP segment displaying a mouse mammary tumor virus (MMTV) protein sequence of the MTV-7 strain was also detected on HAFP domain 1. This segment displayed a 33% identity over a stretch of 27 AA. The significance of these viral-tumor protein sequences to AFP, if any, remains unclear (247). However, it has been reported that tumor (plasmocytoma) formation in mice can be accelerated by treatment with AFP (248, 249). Concomitant with AFP treatment, mice were reported to develop larger tumors, require longer periods of regression, and display higher mortality rates during retrovirus oncogenesis. It is known anecdotally that pregnant women who develop breast cancer during or following pregnancy frequently suffer higher mortality rates in the postnatal months, suggesting an involvement of AFP tumor growth enhancement during gestation (see "AFP and Programmed Cell Death" above).

On the second domain of HAFP, additional disease-related protein identity sites were detected. First, a site for a human Sjögren Syndrome (SS-A/RO, 58 kDa) autoantigen site was found (250), containing a 38% identity over a segment of 16 AA. This autoantigen induces strong antibody titers in both lupus and Sjögren syndrome patients and is germane to this discussion for three reasons. First, the SS-A/RO antigen has been detected in fetal heart tissue and in other organs including fetal, neonatal, and adult skin. The localization of this antigen in fetal myocardium might be related to the elevated serum AFP levels found in pregnancies of fetal heart-block disorders (186). Secondly, anti–SS-A/RO antibodies have been reported to bind to neonatal rabbit cardiac cells and to specifically inhibit cardiac repolarization in cell culture (251). Thirdly, this autoantigen has been demonstrated in biopsies of neonatal and adult skin and was localized at the cell surface of keratinocytes *in situ* (252), whereas AFP is synthesized in skin (114). Further studies indicated that estradiol enhanced the binding of human anti–SS-A/RO antibodies to cultured human keratinocytes (253). This hormone augmentation may explain the sex differences observed in skin manifestation in patients of lupus and Sjögren disorders. As discussed previously,

an AFP-derived 34-mer amino acid sequence has been identified and synthesized which has estrogen regulatory properties (92).

A second site on domain 2 displaying a human Huntington's Disease (HD) peptide repeat was detected, revealing a 40% identity over a 15-AA length. This HD peptide represents an expanded polyglutamate repeat present in all normal brain neurons (254). A Huntington-associated protein binds to the expanded repeat sequence in brain regions afflicted with HD (255). The polyglutamates are thought to interact with glyceraldehyde-3-phosphate dehydrogenase, resulting in reduced energy metabolism of the brain. Interestingly, domain 3 of HAFP did not display any disease-related peptide stretches. The significance of these computer data–derived observations, if any, remains unanswered.

Summary of Computer-Derived Identity Sites on HAFP Domains

Table XIV represents a summary chart of multiple presumptive Genbank computer-generated sequence identities detected on HAFP domains. It is clear at the onset that the three domains of HAFP differ in their sequence identities from these proposed protein groups. A few of the protein groups show identities on all three domains, such as serine protease inhibitors, the T-cell receptors, and fatty acid–binding sites. A second group has sequence identity sites on two of the three domains of HAFP. These are represented by serine proteases, transcription factors, bilirubin binding, MHC (class II), heavy-metal binding, and Ig heavy chains. In other instances, certain domains are totally lacking in sequence identities shared by others. For example, domain 3 lacks a sequence identity site for bilirubin, heavy metals, and lectins, but displays identity sites for cell adhesion proteins, steroid/retinoid binding, homeodomains, DES, and warfarin binding, and acetylcholine receptors. It may be noteworthy that certain groups of matched sequences were found associated with other types of protein classes. For example, domain 3 exhibited cell adhesion proteins together with homeodomain-associated proteins; both protein classes are known to be important for adhesion events during growth and differentiation of the embryo/fetus. Domain 2, for example, displayed a serotonin receptor-like site near a benzodiazopine receptor segment. In comparison, domain 1 bristled with growth factor–related segments such as chorionic somato-mammotiopin, c-*erb* A-β thyroid receptor, transcription factors, and platelet-derived growth factor receptors. Domain 2, based on sequence identities, might be involved with extracellular matrix attachment, lectin, bilirubin, fatty acid and heavy-metal binding, and actin interaction. Domain 3 was also unique in that it lacked certain sequence identities (ATP/tyrosine kinases, and MHC-associated proteins) but solely displayed others (retinoid/steroid binding and heat-shock cognate-70 [HSP-70] proteins).

Domain 1 represents an interesting case in lieu of the AFP mRNA gene expression discussed above for adult AFP in both rodents and humans (see "Ontogenetic AFP Gene Expression" above). According to reports documented in that section, AFP gene translated products were synthesized in which the entire domain 1 and the first 20% of domain 2 were absent. On the basis of the presumptive amino acid sequences presented in Table XIV, one could speculate that a translated protein product of such gene expression might not be involved in certain activities. For example, a truncated adult AFP molecule lacking domain 1 might not be involved in the proposed activities associated with transcription factors, myelin interactions, kainic acid–binding proteins, growth enhancement, lectin binding, and apoptosis. Thus, the major adult form of AFP (37–42 kDa) might be predicted as incapable of growth enhancement as the full-length protein (70 kDa) appears to be. However, a tumor-suppressor sequence might also be absent, which could contribute to a lack of AFP involvement in tumor abrogation in adults.

Concluding Remarks

At the start of this review/prospectus, it was stated that the biological roles of AFP have remained an enigma for many years. The *in vitro* carrier/transport ligand-binding function has proven the easiest to demonstrate. A growth-regulatory role for AFP, albeit may be indirect, can no longer be ignored or denied. The immunoregulatory properties of AFP must await further clarification pending investigational studies employing recombinant AFP and its domains, subdomains and/or fragments. In the author's opinion, AFP is hardly a fetal substitute for albumin since ALB is present in nearly all vertebrate classes together with α-ALB, but true AFP resides only in birds and mammals. Only two short identical stretches are common to all AFPs and ALBs studied (256). One is in domain 2 (16 AA long) and the other is in domain 3 (17 AA long). The domain 1 site resides in a region of platelet-related proteins, while the domain 3 site is localized in an area with high homology with glutathione peroxidase (58% over 31 AA). Clearly, both regions appear to display albumin-related functions.

The present treatise was intended to focus on AFP as a biologic response modifier in order to expand the reader's perspective regarding the functional roles of AFP. By employing a repository of AFP publications in the biomedical literature, attempts were made to reconcile recent computer sequence analyses of AFP domain, subdomain, and motifs/cassettes with these previously published reports. Although the computer findings did not always have a published counterpart, inroads were established to provide both a rationale and justification for further studies of AFP physiology based on domain and subdomain structure. More intense investigation of the functional roles of AFP should prove mutually beneficial to the biomedical community as well as the biotechnology industry. The separation, synthesis, and recombinant production of AFP subdomains and fragments could provide a novel source of unique or modi-

Table XIV. Presumptive Genbank-Derived Protein Sequence Identities to Human α-Fetoprotein (HAFP) Subdivided and Categorized by Domain Location

HAFP domain 1	HAFP domain 2	HAFP domain 3
1. Fibrin-associated protein	Serine proteases	Serine proteases
2. Protease inhibitors	Protease inhibitors	Protease inhibitors
3. —	Extracellular matrix proteins	—
4. —	—	Cell adhesion proteins
5. Microfibril/motor filaments	Actin-related filaments	—
6. Intermediate filaments	—	—
7. Transcription factors	Transcription factors	—
8. Tumor-suppressor proteins	—	—
9. Homeodomain proteins	Homeodomain proteins	Homeodomain proteins
10. Fatty acid binding	Fatty acid binding	Fatty acid binding
11. Bilirubin binding	Bilirubin binding	—
12. —	—	Retinoid binding
13. —	—	Steroid binding
14. —	—	DES, warfarin binding
15. Heavy-metal binding	Heavy-metal binding	—
16. —	Drug binding	Drug binding
17. —	Actin binding	—
18. MHC proteins (Class II)	MHC proteins (Class I, II)	—
19. T-cell receptor protein	T-cell receptor protein	T-cell receptor protein
20. Ig receptor	—	Ig receptor
21. —	Ig heavy chain	Ig heavy chain
22. Growth enhancement	—	Growth suppression
23. Lectin binding	Lectin binding	—
24. —	—	Ach receptor
25. Ca^{2+} binding	Ca^{2+} binding	Ca^{2+} binding

Note. Such sites on AFP might serve as potential binding (dimerizing) sites or areas of molecular mimicry.

fied pharmaceutical derivatives of AFP fragments for testing in such diverse fields as endocrinology, hematology, immunology, and neurology.

If only a small proportion of the amino acid sequence identities presently detected on AFP have any real biological relevance, this would still provide a significant contribution to the physiology of AFP. For example, if the Genbank-detected sequences were present only in the pre- or pro- form of the protein, then AFP might have the potential of binding or dimerizing with the precursor form of that molecule. In theory, this might suppress formation of the mature protein, thereby depleting its intracellular concentration. As presented in a previous review by the author, AFP probably performs different functions at various time points during development (5). Conceptually, the binding of various ligands to AFP during the progressive stages of ontogenetic development might dictate the specific function of AFP at that particular time. AFP function could well be determined by various environmental factors in the body's internal milieu such as ligand concentration, hyper- or hypoxia, pH state, blood sugar (glucose) concentrations, blood-gas partial pressure, osmolality, and plasma alcohol content. Such conditions are known to produce conformational transition forms in AFP that alter tertiary shape and form (7). Many conformational changes are known to be reversible and gradual; AFP then reverts to its original form (7). During these transitional changes, sequence sites concealed in molecular crevices on AFP might be exposed during the environmental stress period and then subsequently resume their hidden state following protein refolding. In this manner different amino acid sequence stretches normally unavailable might be exposed at different times dependent on the environmental shock or distress during that particular interval. AFP might contain multiple molecular hidden sites in reserve to meet a variety of developmental challenges to the fetal environment. Hence, AFP could be "a protein for all seasons" of ontogenetic development.

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