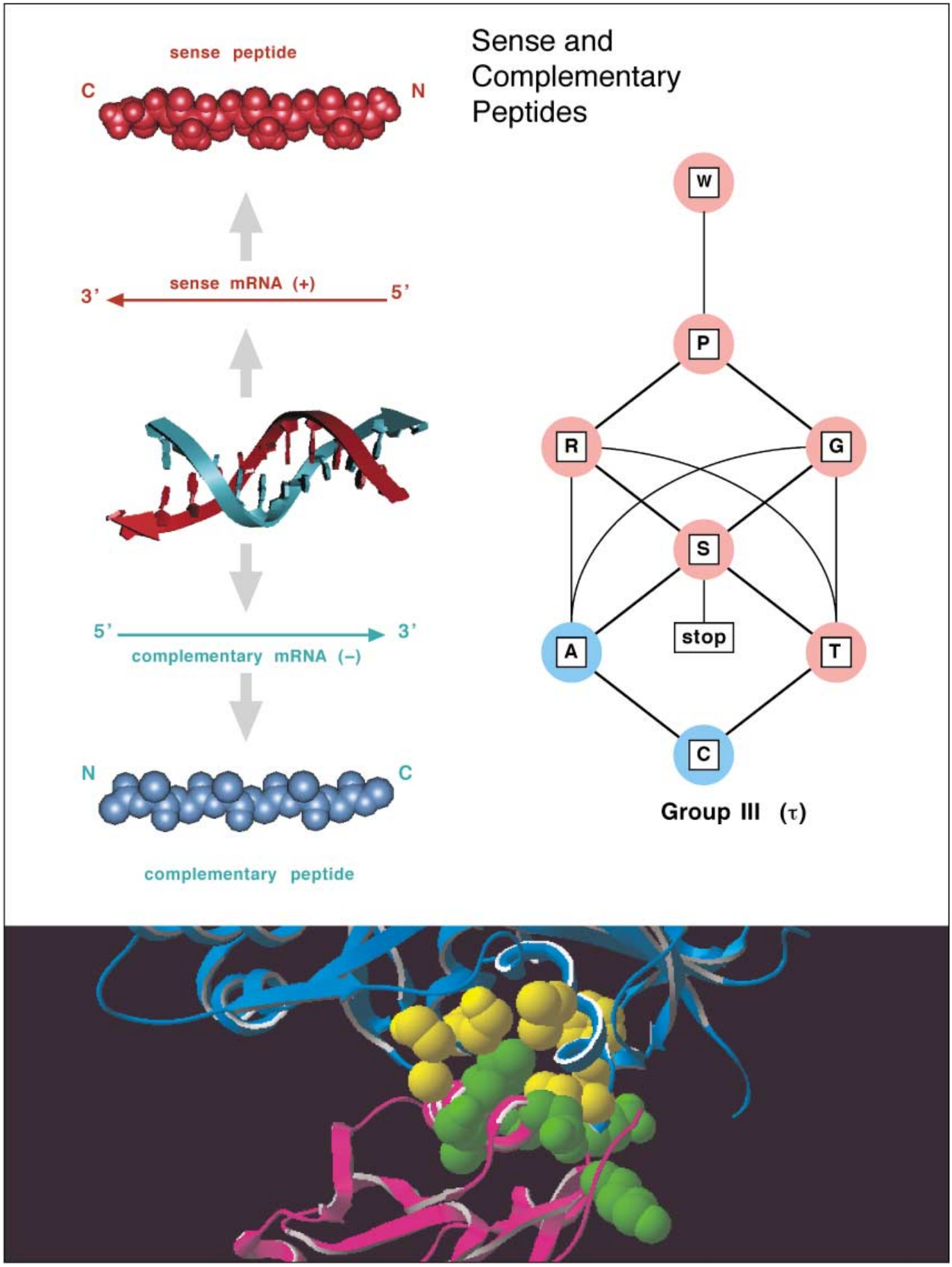

  1439-4227/02/03/02-03 $ 17.50+.50/0 ChemBioChem **2002**, 3, 136 – 151

Specific Interactions Between Sense and Complementary Peptides: The Basis for the Proteomic Code

Jonathan R. Heal,[b] Gareth W. Roberts,[b] John G. Raynes,[c] Ashish Bhakoo,[a] and Andrew D. Miller*[a]

The discovery of the genetic code was one of the milestone events in biology: a conserved, universal code defining the primary amino acid sequences of all proteins of all organisms. However, this code has been thought to be limited, unable to provide additional information appropriate to defining the three-dimensional structure and function of these proteins. This raises important questions. Can there be more to the genetic code? Is there a code embedded within the code? Does a two-dimensional genetic code exist? In our view, the answer to all three of these questions is a qualified "yes". This review describes how sense and complementary peptides coded for by mutually complementary nucleic acid sequences are capable of interacting specifically, thereby suggesting the existence of a second, two-dimensional genetic code (proteomic code). Theories attempting to explain such specific interactions between sense and complementary peptides are discussed including the Mekler–Idlis (M-I) pair theory that suggests that each codon-directed amino acid residue in a sense peptide may make a specific pair-wise interaction with the corresponding complementary codon-directed residue in the complementary peptide. In effect, through-space interactions between pairs of amino acid residues are suggested as being specified by the genetic code and its complement. The biological implications of sense/complementary peptide interactions are potentially vast but still to be fully understood and appreciated. That such peptide/peptide interactions could provide the basis for understanding and constructing the proteomic code remains to be properly established but research to date suggests that we should be able to make a start in that direction.

KEYWORDS:

amino acids · antisense agents · complementary peptides · molecular recognition · proteomics

1. Introduction

The genetic code determines the amino acid sequence of any protein or peptide from the sequence of purine and pyrimidine bases in mRNA transcribed from a strand of double-helical DNA. The linear sequence of purine and pyrimidine bases in mRNA is grouped into nonoverlapping, consecutive sets of three bases (codon), and each of these individual codons usually codes for a single amino acid residue. One-dimensional nucleic acid residue sequence information is translated into one-dimensional amino acid residue sequence information. However, the biological activity of proteins is intimately dependent upon their ability to form defined three-dimensional structures as a result of non-covalent interactions between their constituent amino acid residues. Unfortunately, since the output of the genetic code is only one-dimensional sequences of amino acid residues, there appears to be a significant knowledge gap between the one-dimensional "flat-land" of genes and the three-dimensional world of protein structure and function. This knowledge gap is only now being fully appreciated with the completion of many genome-sequencing projects around the world. Simple genome sequences in themselves do not appear to provide sufficient information to determine the structure and function of the proteome (the total collection of genome-encoded proteins). Therefore, genome sequencing per se appears to have limited value without actual knowledge of the proteins that the genes encode. This has far-reaching implications. For example, there has been a huge investment worldwide into genome sequencing by both pharmaceutical and biotechnology companies alike to try and understand the genetic bases of disease.

According to the traditional view, one strand of DNA, known as the sense (positive) strand, carries the genetic code whilst the complementary or antisense (negative) strand provides the means of propagating that code. This view now appears to be

[a] *Dr. A. D. Miller, A. Bhakoo*
Imperial College Genetic Therapies Centre
Department of Chemistry, Flowers Building, Armstrong Road
Imperial College of Science, Technology and Medicine
South Kensington, London SW7 2AZ (UK)
Fax: (+44) 20-7594-5803
E-mail: a.miller@ic.ac.uk

[b] *Dr. J. R. Heal, Dr. G. W. Roberts*
Proteom Ltd., The Babraham Institute
Babraham, Cambridge CB2 4AT (UK)

[c] *Dr. J. G. Raynes*
Department of Infectious and Tropical Diseases
London School of Hygiene and Tropical Medicine Medicine
London WC1E 7HT (UK)

under threat as evidence is accumulating to show that coding information in the complementary strand of DNA may also be harnessed for transcription.[1] Hence, the relationship between sense and complementary strands of DNA appears to more complex than was at first thought. In addition, there is now a growing pool of evidence to suggest that peptides coded for by sense and complementary strands of DNA can interact specifically in a manner that may be comparable to the way in which sense and complementary strands of DNA interact specifically through Watson–Crick base pairs.[2] These empirical observations of specific interactions between mutually complementary peptides could have profound implications. They suggest the existence of a second, two-dimensional genetic code (proteomic code) that could prove valuable in bridging the gap between genes and proteins, between linear sequence (genetic) and structure/function (proteomic). If generally true, this raises important, wider questions. For instance, are we aware of and do we fully understand all the information embedded in DNA sequences? Can genome sequences per se actually be manipulated to offer all the necessary information to help link linear sequence to three-dimensional structure and function? Without doubt, the existence of the proteomic code could significantly enhance our ability to extract biological value from the very large investment into genome sequencing and associated databases.

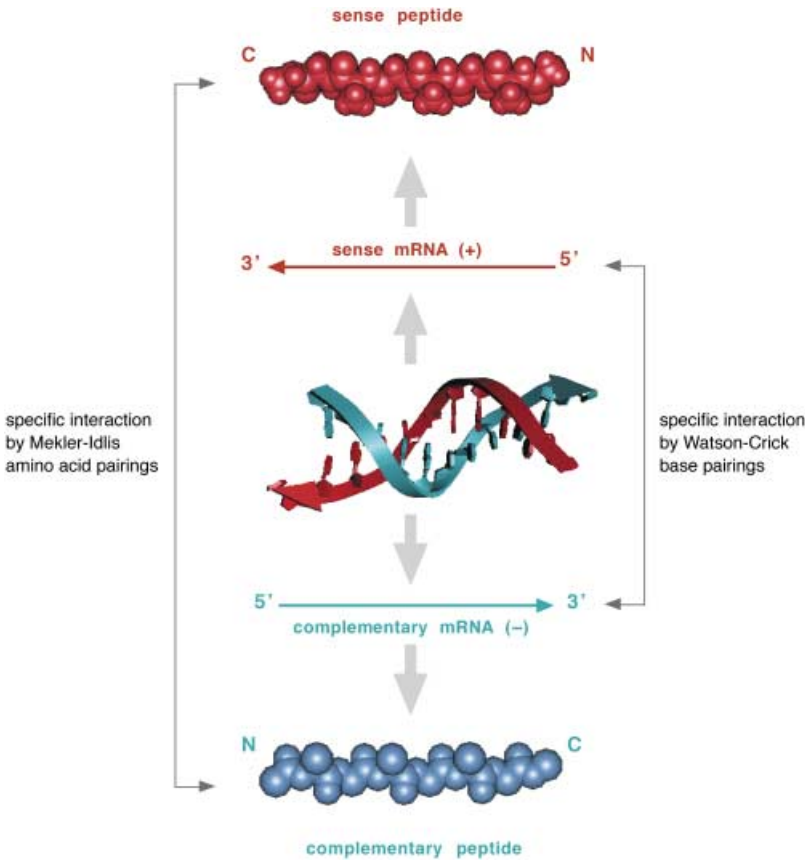

Figure 1. *Illustration to show the basic concept of sense and complementary peptides.*

As we shall illustrate, specific interactions between sense and complementary peptides have been observed and utilised in a wide variety of different biological systems. Moreover, they appear to be important in an impressive array of key molecular recognition processes in nature. Therefore, sense/complementary peptide interactions appear to have real significance and relevance to current bioinformatics, functional genomics, proteomics and protein structure/function analyses. This review will aim to illustrate all this and to show how specific interactions between sense and complementary peptides could provide the basis for understanding and constructing the proteomic code.

2. Interactions between Sense and Complementary Peptides

By definition, a sense peptide is one whose sequence is coded for by the nucleotide sequence (read 5′→3′) of the sense strand of DNA (or by mRNA whose sequence contains the same coding information as the sense strand of DNA). Conversely, the complementary peptide is coded for by the nucleotide sequence (read 5′→3′) of the complementary strand of DNA (or by complementary mRNA with the same sequence information as the complementary strand of DNA). Mekler was the first to suggest that sense and complementary peptides may be able to interact specifically, mediated by specific through-space, pairwise interactions between amino acid residues (Figure 1).[3] According to this suggestion, each codon-directed amino acid

Dr. Andrew David Miller is a Reader in Organic Chemistry and Chemical Biology in the Chemistry Department at Imperial College (London, UK) and is founding Director of the Imperial College Genetic Therapies Centre (GTC). He began his chemistry education at the University of Bristol (UK) from where he graduated in 1984 with a BSc degree. His PhD thesis research was carried out at the University of Cambridge (UK) in the research group of Professor Alan Battersby, after which he joined the research group of Professor Jeremy Knowles at Harvard University (USA). Since 1990, Dr. Miller has been at Imperial College where his group has been researching into the design and testing of nonviral vectors for gene therapy, stress protein chemistry and the proteomic code. In his career, he has received several awards and fellowships. In 2000, he was awarded the Novartis Young Investigator Award in Chemistry and was elected President of the International Society of Cancer Gene Therapy. Dr. Miller cofounded Proteom Ltd. (a GTC spin-out company) in 1999 and is currently the nonexecutive Director.

residue in a sense peptide should be capable of making a specific pair-wise interaction with the corresponding complementary codon-directed residue in the complementary peptide. In effect, Mekler was suggesting that the genetic code and its complement are able to specify through-space interactions between pairs of amino acid residues. Mekler and Idlis identified all of the possible putative interacting pairs of amino acid residues and segregated them into three nonoverlapping groups (Figure 2).[4] In many cases, each given amino acid residue has been partnered by more than one possible amino acid residue. This situation arises because the genetic code is itself degenerate. Therefore, any one amino acid residue may be coded for by up to six different codons matched by the same number of complementary codons that in turn code for up to four alternative matching amino acid residues (Table 1). These Mekler – Idlis (M-I) pairs could represent a protein/peptide equivalent of the Watson – Crick base pairs found in DNA and could not only be the basis of sense/complementary peptide interactions but could also form the foundations of the proteomic code as well. Therefore, these amino acid pairs deserve closer examination.

Within all three groups of M-I pairs, hydrophobic amino acid residues are partnered with hydrophilic amino acid residues and vice versa. This is inevitable given the structure of the genetic code and its complement,[3, 5] as will be described later. Although this may appear somewhat counterintuitive, many of these putative interacting pairs of amino acid residues actually stand up to closer examination. For instance, leucine (Leu, L) and lysine (Lys, K) have been shown to interact spontaneously in aqueous solutions with a significant stabilising ΔG value of $-1.0\ \mathrm{kcal\,mol^{-1}}$; leucine and glutamic acid (Glu, E) interact similarly with a stabilising ΔG value of $-0.5\ \mathrm{kcal\,mol^{-1}}$.[6] In general, hydrophobic interactions between the side chains of nonpolar residues and the hydrocarbon chains supporting the polar functional groups of their putative partner residues would certainly be possible, as observed by Brentani and later by Chaiken.[7, 8] Side chains of hydrophilic amino acids typically consist of a charged or polar functional group connected to a nonpolar alkyl chain. Therefore, pair-wise interactions between amino acid residues of opposite hydropathic character in opposing peptides could be facilitated by stereospecific van der Waals interactions between the hydrophobic components of both side chains. The charged or polar functional group of the more polar residue could then be free to act as a hydrogen-bond donor or acceptor with the adjacent peptide backbone (Figure 3). With this arrangement, the central hydrophobic interactions could create a nonpolar environment, thereby enhancing the strength of the hydrogen bonds formed and assisting the desolvation of the charged or polar functional groups prior to hydrogen-bond formation. Furthermore, Root-Bernstein has noted an alternative side-chain packing arrangement involving glutamate and leucine side chains that also appears to be credible.[9] In addition, we would propose the possibility of weakly polar interactions between the side chains of amino acid pairs based upon shape similarity and complementary charge distribution resulting from normal hyperconjugation and conjugation effects together with σ-bond inductive effects (Figure 4). It is worth noting at this stage that Burley and Petsko have described previously how weakly polar forces should have a strategic importance in protein structure and function.[10]

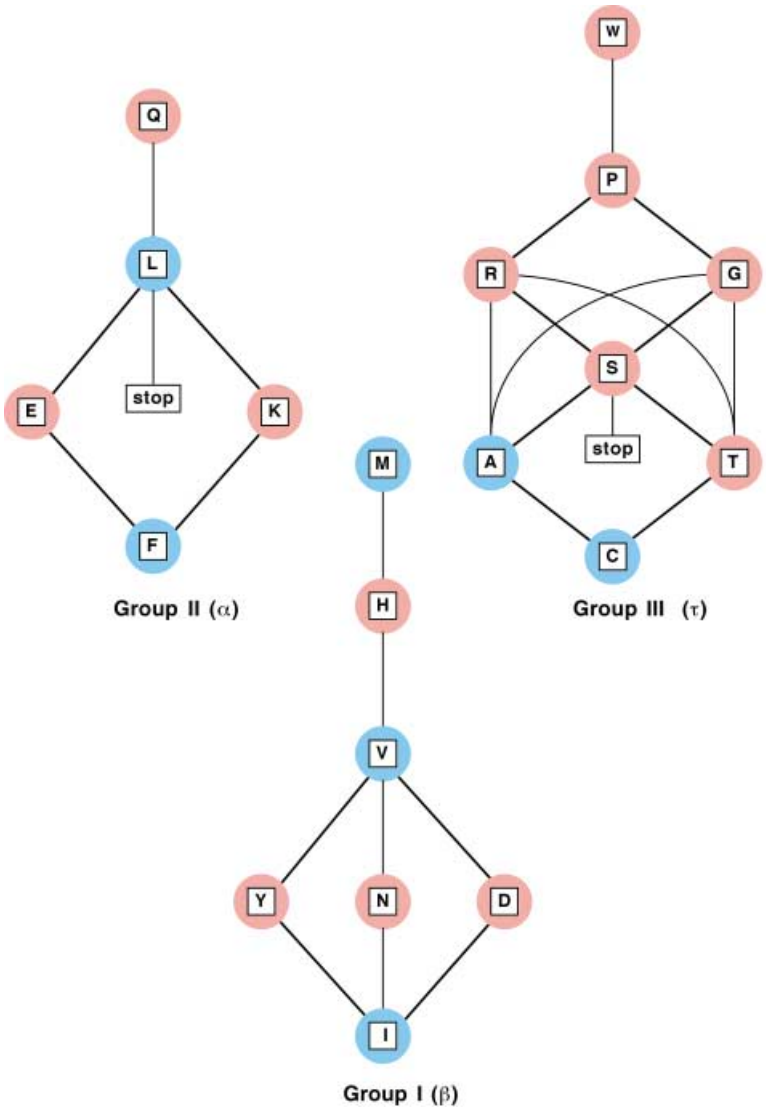

Figure 2. *Schematic representation of the three groups of putative interacting pairs of amino acid residues described by Mekler and Idlis.[4] Amino acids are represented by single-letter codes. "Stop" indicates a stop codon. Solid lines connect codon-directed amino acid residues to their corresponding complementary codon-directed partners with whom they are proposed to form specific through-space pair-wise interactions (see Table 1 for derivation of partners). These M-I pairs are proposed to mediate specific sense/complementary peptide interactions and could represent a protein/peptide equivalent of the Watson – Crick base pairs found in DNA (see Figure 1). Nonpolar residues are shaded blue, polar residues are shaded red. This figure is adapted from the paper by Mekler and Idlis,[4] combined with features adapted from the work of Zull and Smith.[21]*

Table 1. *Table to show how the Mekler – Idlis (M-I) pairs of amino acid residues are derived (see Figure 2).*

Amino acid	Codon 5′ →3′[a]	Complementary codon 5′ →3′[a]	Complementary amino acid	Amino acid	Codon 5′ →3′[a]	Complementary codon 5′ →3′[a]	Complementary amino acid
Ala (A)	GCA	UGC	Cys (C)	Ser (S)	UCA	UGA	stop
	GCG	CGC	Arg (R)		UCC	GGA	Gly (G)
	GCC	GGC	Gly (G)		UCG	CGA	Arg (R)
	GCU	AGC	Ser (S)		UCU	AGA	Arg (R)
					AGC	GCU	Ala (A)
					AGU	ACU	Thr (T)
Arg (R)	CGG	CCG	Pro (P)	Gln (Q)	CAA	UUG	Leu (L)
	CGA	UCG	Ser (S)		CAG	CUG	Leu (L)
	CGC	GCG	Ala (A)				
	CGU	ACG	Thr (T)				
	AGG	CCU	Pro (P)				
	AGA	UCU	Ser (S)				
Asp (D)	GAC	GUC	Val (V)	Gly (G)	GGA	UCC	Ser (S)
	GAU	AUC	Ile (I)		GGC	GCC	Ala (A)
					GGU	ACC	Thr (T)
					GGG	CCC	Pro (P)
Asn (N)	AAC	GUU	Val (V)	His (H)	CAC	GUG	Val (V)
	AAU	AUU	Ile (I)		CAU	AUG	Met (M)
Cys (C)	UGU	ACA	Thr (T)	Ile (I)	AUA	UAU	Tyr (Y)
	UGC	GCA	Ala (A)		AUC	GAU	Asp (D)
					AUU	AAU	Asn (N)
Glu (E)	GAA	UUC	Phe (F)	Leu (L)	CUG	CAG	Gln (Q)
	GAG	CUC	Leu (L)		CUC	GAG	Glu (E)
					CUU	AAG	Lys (K)
					UUG	CAA	Gln (Q)
					UUA	UAA	stop
					CUA	UAG	stop
Lys (K)	AAA	UUU	Phe (F)	Thr (T)	ACA	UGU	Cys (C)
	AAG	CUU	Leu (L)		ACG	CGU	Arg (R)
					ACC	GGU	Gly (G)
					ACU	AGU	Ser (S)
Met (M)	AUG	CAU	His (H)	Trp (W)	UGG	CCA	Pro (P)
Phe (F)	UUU	AAA	Lys (K)	Tyr (Y)	UAC	GUA	Val (V)
	UUC	GAA	Glu (E)		UAU	AUA	Ile (I)
Pro (P)	CCA	UGG	Trp (W)	Val (V)	GUA	UAC	Tyr (Y)
	CCC	GGG	Gly (G)		GUG	CAC	His (H)
	CCU	AGG	Arg (R)		GUC	GAC	Asp (D)
	CCG	CGG	Arg (R)		GUU	AAC	Asn (N)

[a] The codons for each possible amino acid residue of a sense peptide are shown alongside their complementary codons. All alternative interacting partner residues in a 5′ →3′ complementary peptide are identified by reading these complementary codons in the 3′ →5′ direction.

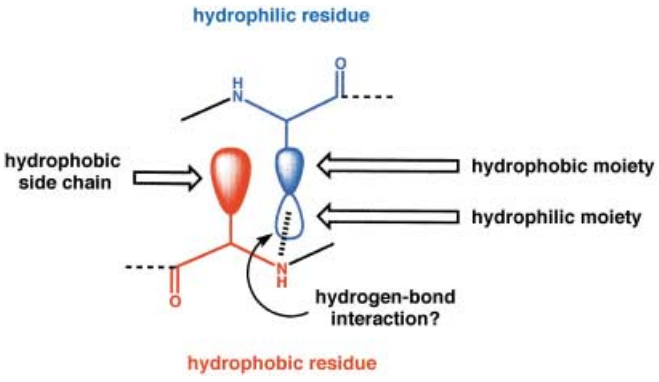

Figure 3. *Model to account for the specific interaction of sense and complementary peptides composed of M-I Group I and II amino acid pairs. This figure is adapted from the paper of Chaiken.*[8]

The specific Group II interactions proposed between glutamate or lysine and phenylalanine (Phe, F) are possible by π-face hydrogen bonding.[9, 10] This mode of interaction would involve the aromatic ring of phenylalanine (hydrogen-bond acceptor) and either the γ-carboxylic acid or the ε-amino groups (hydrogen-bond donors) of glutamic acid or lysine respectively. In the case of Group III amino acid pairs, proline (Pro, P) and glycine (Gly, G) are well known to interact ubiquitously between the polypeptide chains of collagen. Similarly, the side chains of alanine (Ala, A), serine (Ser, S) and glycine juxtapose between the polypeptide chains of fibroin. Furthermore, serine and threonine (Thr, T) are inherently capable of forming a specific hydrogen bond between themselves, as are threonine and cysteine (Cys, C).[9] Even specific interactions between alanine and arginine (Arg, R) appear to be established.[9] Finally, Samanta et al. have

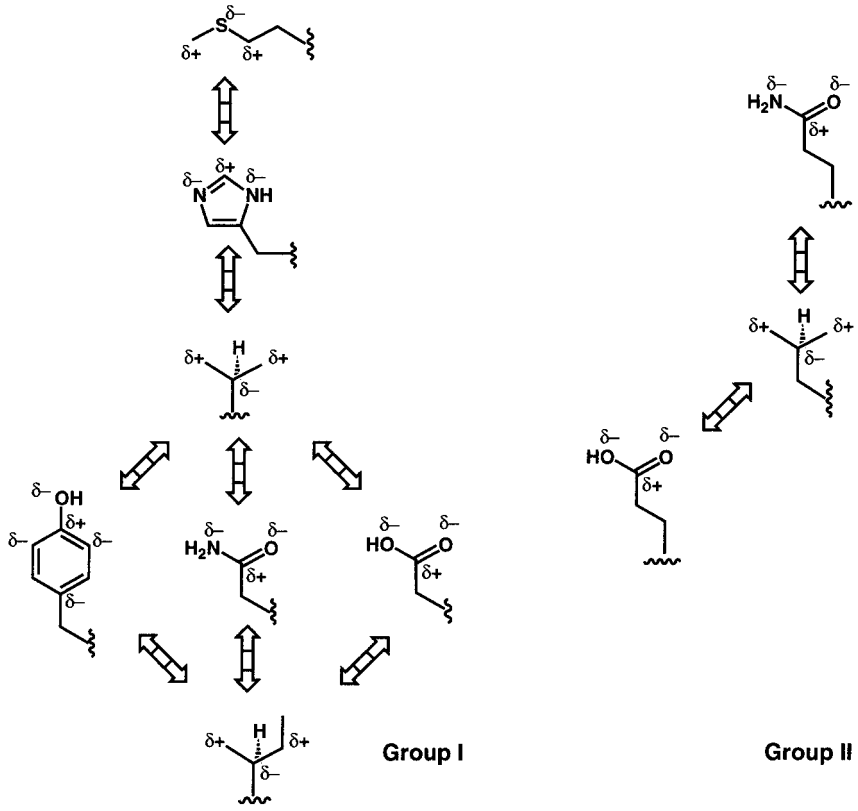

Figure 4. *A representation of a subset of M-I Group I and II amino acid pairs to show how the stereoelectronic properties of the hydrophobic and hydrophilic amino acid residues that comprise each M-I pair could complement each other in order for specific side-chain interactions to take place.*

recently reported the high incidence of proline – tryptophan (Trp, W) contacts in known protein X-ray crystal structures.[11]

The physico-chemical evidence that has been accumulated sporadically over the past few years would appear to support the suggestion that the M-I pairs could be responsible for the specific interactions between sense and complementary peptides. For instance, both experimental and modelling data support the notion that hydrophobic, electrostatic, and hydrogen-bonding interactions are all important in bringing sense and complementary peptides together.[12–16] This is important given the fact that M-I pairs must necessarily associate by means of a similar range of bonding interactions, as indicated above. In addition, the strength of sense/complementary peptide interactions has been shown to increase with length, with longer pairs of sequences usually displaying a higher mutual affinity than shorter pairs.[12, 17] Furthermore, there is significant experimental evidence to suggest that sense/complementary peptide interactions are "conformationally degenerate", multilocalised/multisite and made up of clusters of stabilising, noncovalent contacts between specific amino acid residues.[12, 13, 15, 17] The deleterious consequences of mutations in either a sense or complementary peptide have also been noted, further underlining the importance of specific amino acid contacts in sustaining sense/complementary peptide interactions.[13, 18]

However, if M-I pairs are to form the basis of the specific interaction between sense and complementary peptides, then molecular modelling studies suggest that both peptides would, in fact, need to adopt extended β-strand or P_{II}-helix conformations and align in an antiparallel fashion. In this way, the side chains of each codon and corresponding complementary codon-directed amino acid residue would be able to come into close physical proximity with each other.[9, 16, 19] There is some experimental data available to support this modelling work,[12] including circular dichroism spectroscopy data which suggests that β-sheet structures are formed with interactions between sense and complementary peptides.[15] Consistent with this, some M-I pairs (glycine – alanine, valine (Val, V) – tyrosine (Tyr, Y), serine – threonine and arginine – threonine) have been noted independently to show strong preferences for specific positions in adjacent β-strands of protein β-structures.[20] Finally, it is noteworthy that Zull and Smith have independently described the M-I pairs but focussed on observations that Group I residues show a higher propensity to form β-sheets, Group II to form α-helices and Group III to form β-turns.[21] On the basis of this, they suggested that sense and complementary peptides should have equivalent conformations in terms of their secondary and tertiary structures. Such a suggestion is consistent with the foregoing discussion. For the remainder of this review, the theory of Mekler and Idlis that the specific interaction between a given sense and complementary peptide is mediated by M-I pairs shall be called the M-I pair theory.

3. Complementary Peptides and Hydropathic Complementarity

Blalock and Smith were the first to note that the hydropathic character (measured on the Kyte – Doolittle scale)[22] of an amino acid residue is related to the identity of the middle letter of the mRNA codon from which it is translated.[5] When uridine (U) is the middle nucleoside, the codon always codes for a hydrophobic residue whilst with adenosine (A), the codon codes for a hydrophilic residue. Similarly, codons with either cytidine (C) or guanosine (G) as their middle nucleosides always code for residues with generally similar hydrophilic/hydrophobic characteristics. A and U (equivalent to thymidine (T) in DNA) are complementary nucleosides according to Watson – Crick base pairing rules, as are G and C. Consequently, sense and complementary mRNA must always code for peptide sequences that are opposite in hydropathic character to each other. Blalock rationalised that a given sense and complementary peptide pair should therefore have mutually complementary shapes (secondary and tertiary structures), owing to the "inverse forces" operating within each peptide because of their opposite

hydropathic profiles, and hence should be able to interact specifically.[23] In other words, Blalock was suggesting that hydropathic complementarity creates the basis of the "molecular recognition" code that drives specific interactions between sense and complementary peptides. This has been called the molecular recognition theory (MRT; Figure 5).[2, 23]

Markus et al. attempted to produce evidence in support of the MRT with some modelling work.[19] By analysing the available conformations for a pair of peptides characterised by mutually complementary hydropathic profiles, they concluded that interaction would only be optimal if both peptides were in extended β-strand conformations, forming either parallel or anti-parallel β-ribbons (Figure 6). In this way, the side chains of hydrophobic amino acid residues could be orientated into proximity with each other, leaving the side chains of hydrophilic amino acid residues to project outwards into the surrounding aqueous environment. This model was perceived to have the singular advantage that "inconvenient" pair-wise interactions between hydrophilic and hydrophobic amino acid residue side chains would be completely avoided. Hence, the affinity of a sense peptide for its complementary peptide need only be accounted for as a result of hydrophobic interactions and the hydrophobic effect. However, such a model could only work if both sense and complementary peptides were comprised of alternating series of hydrophilic and hydrophobic amino acid residues. This is not generally the case.

Several experimental investigations have been directed at trying to understand the putative relationship between binding and the hydropathic profiles of sense and complementary peptides. One of the most comprehensive of these was carried out by Fassina et al. who studied the relationship between a thirteen residue section of a glycoprotein and variants of the corresponding complementary peptide designed to maximise the degree of mutual hydropathic complementarity.[17] A positive correlation was observed between improved hydropathic complementarity and binding affinity; this implys that precise hydropathic complementarity is linked to successful interaction. Other groups including ourselves have offered some support for this contention.[12, 13, 15, 18, 24] However, a closer examination of some of the peptide sequences involved is revealing. For instance, Fassina et al. have described a peptide $C.G._{RAF}$ that has the highest possible hydropathic complementarity to the c-Raf protein (residues 356 – 375) and is biologically active.[15] Although obviously different in sequence to the complementary mRNA-derived comple-

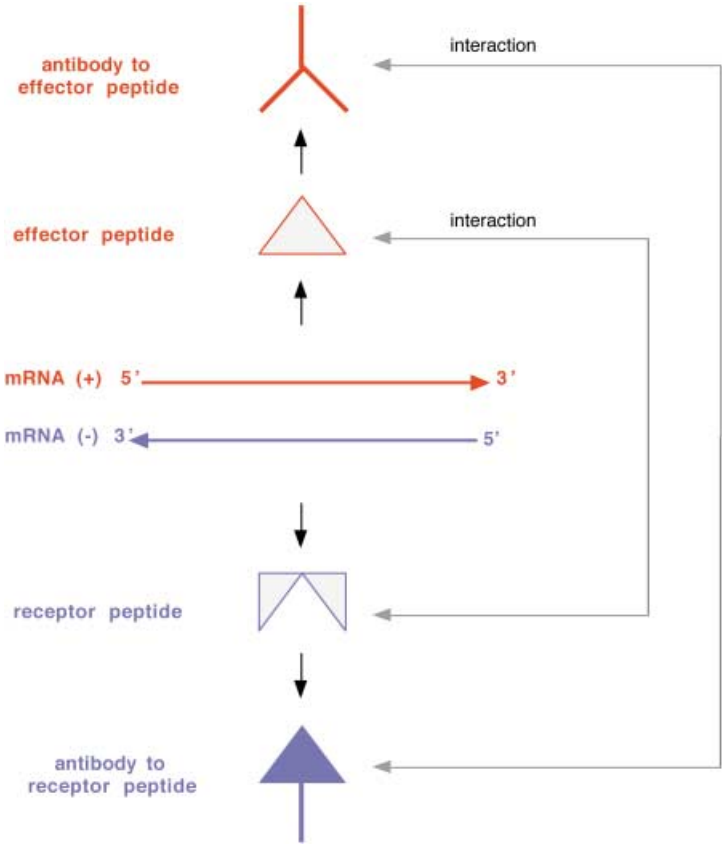

Figure 5. *A diagrammatic representation of the molecular recognition theory (MRT) as proposed by Blalock.[2, 23] The mutually complementary hydropathic profiles of a given pair of sense and complementary peptides are presumed to induce these peptides to adopt mutually complementary three-dimensional shapes capable of interacting specifically with each other. Assuming antibody/antigen interactions to be governed by similar principles, antibodies raised against the sense and complementary peptide in turn should have mutually complementary antigen combining regions and have an idiotypic/anti-idiotypic relationship with respect to each other. The evidence for this is discussed in the text.*

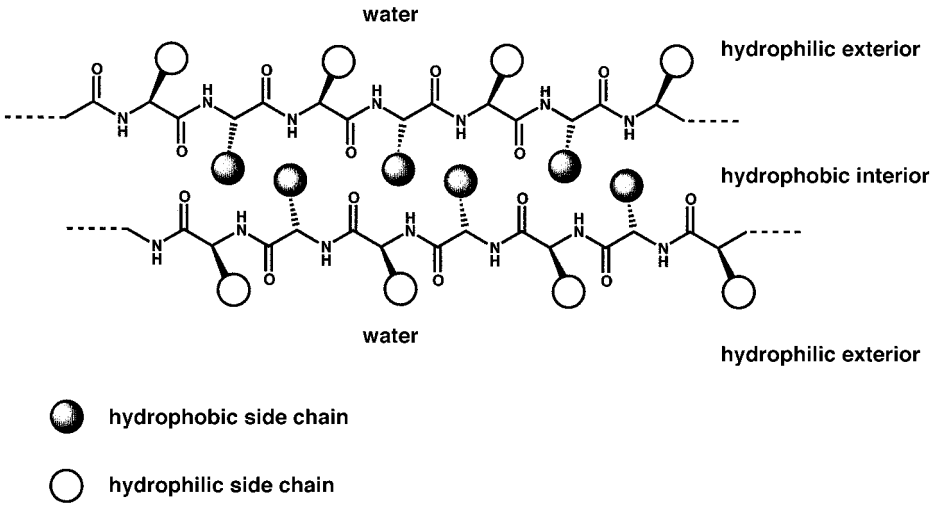

Figure 6. *Model of Markus et al. proposing how sense and complementary peptides might align in order to "optimise" binding interactions.[19] Filled grey circles represent hydrophobic side chains, whilst empty circles represent hydrophilic side chains. This alignment was proposed to avoid "inconvenient" pair-wise interactions between hydrophilic and hydrophobic amino acid residue side chains. However, such a model could only work if both sense and complementary peptides were comprised of alternating series of hydrophilic and hydrophobic amino acid residues. This is not generally the case.*

mentary peptide AS_{RAF}, each residue of $C.G._{RAF}$ belongs not only to the same M-I group as the corresponding residue in AS_{RAF} but is also a recognised alternative in terms of allowed M-I group pairings (see Figure 2 and Table 1). Similarly, Sisto has described a peptide that has the highest possible hydropathic complementarity to the β-bulge trigger loop region of interleukin-1β (IL-1β) (residues 47 – 55 (mature sequence)) and is biologically active.[25] Once again, although obviously different in sequence to the complementary mRNA-derived peptide,[26] each corresponding residue of the Sisto peptide, bar two, does belong to the same M-I group and is also a recognised alternative in terms of allowed M-I group pairings (see Figure 2 and Table 1). Things are not always as clear-cut as this. In some other cases, complementary peptide sequences derived on the basis of mutual hydropathic complementarity do not appear to coincide with the M-I groups in such a straightforward way.[24] However, such peptides can be encompassed if hydrophobic amino acid residues such as leucine, isoleucine and valine are regarded as stereoelectronically equivalent to each other, on the basis of their closely related side-chain structures, and are able to surrogate for each other within M-I Groups I and II (Figure 2). Therefore, we are now of the opinion that hydropathic complementarity per se, and by implication the MRT, is not responsible for the interaction between sense and complementary peptides. Instead the concept provides an alternative, more empirical and, in our view rather misleading, description of the M-I pair theory. This is important. Since the inception of the MRT, the M-I pair theory appears to have been largely ignored, with preference given to the MRT. In our opinion, this is a grave oversight that should be corrected from now on. The main differences between the M-I pair theory and the MRT are summarised (Table 2).

4. Alternative Complementary Peptides

The definition of complementary peptides has been widened in some quarters to include peptides whose sequence is determined by reading the nucleoside sequence of complementary mRNA in the alternative 3′ →5′ direction.[27] They noted that a 3′ →5′ complementary peptide should have a similar hydropathic profile to a 5′ →3′ complementary peptide given the fact that it is only the middle nucleoside base of a codon or complementary codon that defines the hydropathic character of an amino acid residue (see above). Therefore, a 3′ →5′ complementary peptide could interact with a sense peptide in an equivalent manner to a 5′ →3′ complementary peptide, on the basis of the MRT. Alternatively, an interpretation based upon M-I pair-wise interactions is still possible. When complementary codons are read in the 3′ →5′ direction, the impact of the degeneracy of the genetic code becomes markedly reduced (Table 3).[9, 28] At the same time though, many of the resulting Root-Bernstein (R-B) amino acid residue pairs are still the same as the original M-I pairs (Table 1). Moreover, if we allow leucine, isoleucine and valine to be interchangeable as described above, then only three R-B pairs are definitively different from the original M-I pairs. These are methionine (Met, M) and tyrosine, tryptophan and threonine, and a serine – serine pair (compare Tables 1 and 3). Therefore, the R-B pairs can be regarded as a subset of the M-I pairs. Hence, the specific interactions observed between sense and 3′ →5′ complementary peptides are likely to be guided by the same M-I pair theory principles as interactions between sense and 5′ →3′ complementary peptide interactions. There would need to be one main difference though. Root-Bernstein has noted that extended conformations of sense and 3′ →5′ complementary peptides should align in a parallel fashion (the opposite orientation) in order for the side chains of each codon and 3′ →5′ complementary codon-directed amino acid residue to interact properly.[9]

Complementary peptides may still be derived even if sense nucleic acid sequences are not known. For instance, the nucleotide sequence of sense mRNA coding for a sense peptide of interest may be approximated by using preferred codon usage tables,[29] allowing first the sequence of complementary mRNA and second the preferred complementary peptide sequence to be deduced (Tables 1 and 3). Alternatively, the sequence of a complementary peptide may be deduced directly from the sequence of a sense peptide by using a compilation of the "most probable" complementary amino acid residues (Tables 1 and 3). For example, leucine may be coded for by one of six possible codons. However, of the six complementary codons, two could code for glutamine, one for glutamic acid and one for lysine, not to mention two that could code for "stop" (Table 1). Therefore, in this case, the "most probable" complementary amino acid residue for leucine would be glutamine. Methods of these types have been employed with some success.[12, 13, 17, 30, 31]

Table 2. *Summary of the main characteristics of M-I pair theory and MRT proposed to account for the specific interactions between sense and complementary peptides.*

M-I pair theory	MRT
based on sense and complementary genetic code	based on biophysical property of hydropathy
sense and complementary peptides interaction mediated by specific through-space interactions between pairs of amino acid residues (M-I pairs) specified by the genetic code and its complement	sense and complementary peptides have mutually complementary hydropathic profiles and therefore mutually complementary shapes owing to "inverse forces" operating within each peptide.
M-I pairs could be peptide/protein equivalent of Watson – Crick base pairs found in DNA	complementary shapes are the basis of specific interactions ("lock and key")
extended conformation required for interactions	unspecified secondary and tertiary structures required for interactions
variety of evidence for M-I pair interactions involving extended peptide conformations	actual evidence for MRT appears to be ambiguous
	MRT can be shown to be an empirical description of the M-I pair theory

Table 3. *Table to show how the Root-Bernstein (R-B) pairs of amino acid residues are derived.*

Amino acid	Codon 5′→3′[a]	Complementary codon 3′→5′[a]	Complementary amino acid	Amino acid	Codon 5′→3′[a]	Complementary codon 3′→5′[a]	Complementary amino acid
Ala (A)	GCA	CGU	Arg (R)	Ser (S)	UCA	AGU	Ser (S)
	GCG	CGC	Arg (R)		UCC	AGG	Arg (R)
	GCC	CGG	Arg (R)		UCG	AGC	Ser (S)
	GCU	CGA	Arg (R)		UCU	AGA	Arg (R)
					AGC	UCG	Ser (S)
					AGU	UCA	Ser (S)
Arg (R)	CGG	GCC	Ala (A)	Gln (Q)	CAA	GUU	Val (V)
	CGA	GCU	Ala (A)		CAG	GUC	Val (V)
	CGC	GCG	Ala (A)				
	CGU	GCA	Ala (A)				
	AGG	UCC	Ser (S)				
	AGA	UCU	Ser (S)				
Asp (D)	GAC	CUG	Leu (L)	Gly (G)	GGA	CCU	Pro (P)
	GAU	CUA	Leu (L)		GGC	CCG	Pro (P)
					GGU	CCA	Pro (P)
					GGG	CCC	Pro (P)
Asn (N)	AAC	UUG	Leu (L)	His (H)	CAC	GUG	Val (V)
	AAU	UUA	Leu (L)		CAU	GUA	Val (V)
Cys (C)	UGU	ACA	Thr (T)	Ile (I)	AUA	UAU	Tyr (Y)
	UGC	ACG	Thr (T)		AUC	UAG	stop
					AUU	UAA	stop
Glu (E)	GAA	CUU	Leu (L)	Leu (L)	CUG	GAC	Asp (D)
	GAG	CUC	Leu (L)		CUC	GAG	Glu (E)
					CUU	GAA	Glu (E)
					UUA	AAU	Asn (N)
					CUA	GAU	Asp (D)
					UUA	AAC	Asn (N)
Lys (K)	AAA	UUU	Phe (F)	Thr (T)	ACA	UGU	Cys (C)
	AAG	UUC	Phe (F)		ACG	UGC	Cys (C)
					ACC	UGG	Trp (W)
					ACU	UGA	stop
Met (M)	AUG	UCA	Tyr (Y)	Trp (W)	UGG	ACC	Thr (T)
Phe (F)	UUU	AAA	Lys (K)	Tyr (Y)	UAC	AUG	Met (M)
	UUC	AAG	Lys (K)		UAU	AUA	Ile (I)
Pro (P)	CCA	GGU	Gly (G)	Val (V)	GUA	CAU	His (H)
	CCC	GGG	Gly (G)		GUG	CAC	His (H)
	CCU	GGA	Gly (G)		GUC	CAG	Gln (Q)
	CCG	GGC	Gly (G)		GUU	CAA	Gln (Q)

[a] The codons for each possible amino acid residue of a sense peptide are shown alongside their complementary codons. All alternative interacting partner residues in a 5′→3′ complementary peptide are identified by reading these complementary codons in the 3′→5′ direction.

5. Observations with Sense/Complementary Peptide Systems

Jones was the first to perform experiments using sense and complementary peptides.[32] The complementary peptides that he produced were designed to bind to the C-terminal tetrapeptide of gastrin. All were reported to be biologically inactive with the exception of the only 5′→3′ complementary peptide that showed some antagonism to gastrin in vivo. Subsequent investigations of Blalock and co-workers represent the next explicit experimental examination of sense/complementary peptide interactions.[27] In their studies, a high-affinity association was reported between the naturally occurring adrenocorticotropin hormone (ACTH) peptide and a synthetic 5′→3′ complementary peptide whose sequence had been deduced from the nucleotide sequence of complementary mRNA (reading 5′→3′). The same research group later found a similar high-affinity association between the γ-endorphin peptide and a synthetic 5′→3′ complementary peptide whose sequence had been deduced in the same way.[33] Since then, numerous independent experimental studies have been reported in which complementary peptides have been derived, usually from complementary DNA or mRNA sequences, for a variety of alternative applications (Table 4). Some of these applications will be discussed in a little more detail below. Several of these experimental studies have involved investigations with 3′→5′ complementary peptides. These include studies with complementary peptides to adrenocorticotropin hormone (ACTH),[34] angiotensin II (AII),[35, 36] arginine vasopressin (AVP),[37, 38] von Willebrand factor (vWf) and vitronectin (Vn),[39] fibrinogen,[40] growth hormone releasing hormone (GHRH),[41] luteinising hormone releasing hormone (LHRH),[42] melanocyte stimulating hormone

Table 4. *Main biological protein/peptide systems from which complementary peptides have been derived and utilised.*

Sense peptide/protein system	Reference
acetyl choline receptor	Araga et al.[100]
adrenocorticotropin	Bost et al.[27]
	Blalock and Bost[34]
anaphylatoxin C5a	Baranyi et al.[108]
angiogenin	Gho and Chae[66]
angiotensin II	Elton et al.[97]
	Soffer et al.[35]
	Moore et al.[116]
	Holsworth et al.[36]
arginine vasopressin	Johnson and Torres[37]
	Knigge et al.[117]
	Lu et al.[112]
	Kelly et al.[137]
β-endorphin	Shahabi et al.[118]
big endothelin	Fassina et al.[63],
	Fassina et al.[55]
calcium binding domain	Dillon et al.[59]
c-Raf protein	Fassina et al.[15]
cystatin-C	Ghiso et al.[68]
dopamine receptor	Nagy and Frawley[119]
endothelin receptor	Baranyi et al.[92]
	Wu et al.[120]
[Met]-enkephalin	Carr et al.[121]
	Misra et al.[122]
	Stambuk et al.[123]
fibrinogen	Gartner and Taylor[40]
fibronectin	Brentani et al.[98]
	Pasqualini et al.[124]
γ-endorphin	Carr et al.[33]
	Carr et al.[135]
	Martin-Moe et al.[60]
gastrin terminal peptide	Jones[32]
	McGuigan and Campbell-Thompson[125]
growth hormone releasing hormone	Grosvenor and Balint[126]
	Weigent et al.[41]
gp41 of HIV	Imai et al.[127]
anti-idiotypic antibodies	Bost and Blalock[30]
	Blalock et al.[128]
insulin	Knutson[129]
integrin	Derrick et al.[130]
interferon-γ	Johnson et al.[131]
interferon-β	Scapol et al.[114]
interleukin-1	Fassina and Cassani[62]
	Davids et al.[26]
	Sisto[25]
	Heal et al.[24]
interleukin-2	Weigent et al.[132]
	Fassina et al.[113]
laminin receptor	Castronovo et al.[133]
luteinising hormone releasing hormone	Mulchahey et al.[96]
	Root-Bernstein and Westall[42]
melanocyte stimulating hormone	Al-Obeidi et al.[43]
trypsin-modulating oostatic factor	Borovsky et al.[46]
myelin basic protein	Zhou and Whitaker[105]
neurophysin II	Fassina et al.[14]
	Fassina[65]
neutrophil chemoattractant	Pfister et al.[109]
nitric oxide synthase	Sautebin et al.[47]
ovine prolactin	Bajpai et al.[134]
P2 protein	Araga et al.[104]
prion protein	Martins et al.[110]
ribonuclease S-peptide	Shai et al.[12]
	Shai et al.[13]
somatostatin	Campbell-Thompson and McGuigan[136]
substance P	Pascual et al.[44]
	Bret-Dibat et al.[45]
T15 self-binding antibody	Kang et al.[101]
tumour necrosis factor-α	Fassina et al.[64]
vitronectin	Gartner et al.[39]

(MSH),[43] Substance P (SP),[44, 45] trypsin modulating oostatic factor (TMOF)[46] and nitric oxide synthase.[47]

Specific sense/complementary peptide interactions have not always been found in all the various systems in which they have been sought. Nor have all the examples mentioned above necessarily been reproduced successfully by the efforts of other research groups.[48, 49] Examples of systems where interactions have not been observed reproducibly include the angiotensin II (AII),[50–52] parathyroid hormone[53] and insulin-like growth factor I (IGF-I) systems.[54] There is, as yet, no clear explanation as to why specific sense/complementary peptide interactions should not be observed reproducibly in these experiments whilst being seen with such apparent ease in others. These inconsistencies must be resolved if sense/complementary peptide interactions are to be fully appreciated and understood.

5.1. Physical evidence for interactions between sense and complementary peptides

In general, it has proved quite difficult to obtain clear structural information about sense/complementary peptide interactions in solution. By using ^{1}H NMR spectroscopy, Fassina et al. were able to detect changes in the chemical shifts of aromatic, aliphatic and backbone amide protons of big endothelin (residues 16 – 29) and its complementary peptide consistent with a specific binding interaction in solution.[55] Root-Bernstein and Westall have also described similar effects when studying mixtures of either fibrinopeptide A or luteinising hormone releasing hormone (LHRH) and their corresponding complementary peptides.[42, 56] These chemical shift changes were informative but could not be ascribed to a particular mechanism or structural state of binding. However, when parallel ^{1}H NMR studies were conducted with either adrenocorticotropin (ACTH) or angiotensin II (AII) and their respective complementary peptides, no such chemical shift changes were observed.[49, 52] By contrast, some electrospray mass spectrometry studies have been able to suggest that angiotensin II (AII) and complementary peptides are able to interact to form noncovalent heterodimeric complexes with μM dissociation constants.[57] Similar observations have been made in mass spectral studies of [Met]-enkephalin and [Leu]-enkephalin interacting with their respective complementary peptides.[58]

Like ^{1}H NMR spectroscopy, circular dichroism (CD) spectroscopy studies have also proved a little contradictory. Fassina et al. reported that the CD spectrum of a mixture of big endothelin and its complementary peptide was different from a simple addition spectrum formed from a linear combination of the individually recorded spectra of each peptide.[55] Furthermore, Dillon et al. have reported the formation of β-strand conformations following the interaction of a Ca^{2+} ion peptide mimetic, complementary to calmodulin-like Ca^{2+} ion binding sites,

with Ca^{2+} ion binding agents.[59] Similarly, CD spectral data generated by Fassina et al. does give the impression that β-sheet structures may be formed in solution following an interaction between sense and complementary peptides.[15] However, comparable CD studies conducted with adrenocorticotropin (ACTH) or γ-endorphin and their respective complementary peptides revealed no such spectral changes consistent with binding interactions.[49, 60]

Curto and Krishna have tried to explain the variability in ^{1}H NMR spectroscopy results by suggesting that sense peptides in solution, being highly flexible, need to overcome a large conformational entropy barrier before they may recognise and bind to the "proper" conformation of the complementary peptide partner.[61] Such a process is clearly difficult for a pair of mobile peptides to achieve in free solution. Given this explanation it is clear that, were one of the components to be immobilised, constraining motion and conformation, encounter frequencies between sense and complementary peptides in the "proper" conformation should be significantly increased and interactions observed more readily. This is indeed what appears to happen. Interactions between sense and complementary peptides are readily and reproducibly observed when one component is immobilised on a solid-phase matrix. Techniques such as affinity chromatography[12, 13, 55, 62–65] and resonant mirror/surface plasmon resonance biosensing[18, 24, 26] have proven particularly successful in this regard. So indeed have enzyme-linked immunosorbent assay (ELISA) methods.[66] By using these techniques, specific dissociation constants, K_d, in the μM – nM range have been measured routinely.[12, 15, 24, 26, 66]

5.2. Receptor/ligand binding mediated by sense/complementary peptide interactions

There is now a reasonable body of evidence to suggest that sense/complementary peptide interactions may play strategic roles in guiding the association between peptide/protein ligands and their respective receptors. Bost et al. provided experimental evidence for this by showing that the interactions between three different protein effectors (interleukin-2, epidermal growth factor and transferrin) and their respective receptors could all be mediated by mutually complementary peptide sequences coded for by mutually complementary effector and receptor mRNA sequences.[67] Subsequently, Ghiso et al. suggested that the interaction between cystatin C, a cysteine proteinase inhibitor, and the fourth component of complement C4 might also involve peptide segments coded for by mutually complementary cystatin C and C4 DNA sequences.[68] Even more tantalising still, Ruiz-Opazo et al. recently reported how screening a complementary DNA (cDNA) library by using antisense oligonucleotides corresponding to angiotensin II (AII) and arginine vasopressin (AVP) led to the isolation of a novel, dual angiotensin II/vasopressin receptor gene.[69] Site-directed mutagenesis studies clearly established that corresponding sense and complementary peptide sequences in receptor and ligands were responsible for mediating the interactions between them.

There have been other reports corroborating the importance of sense/complementary peptide regions in mediating receptor/ligand interactions. These include the binding of human follicle stimulating hormone (hFSH),[70] human erythropoietin (hEPO)[71, 72] and angiotensin II (AII)[73] to their respective receptor proteins. Interactions between peptide hormones and G-protein coupled receptors may be also governed by mutually complementary peptide sequences in hormones and respective receptors, in general.[71] Even the diverse interactions of neurokinin A (NKA) with NK1 tachykinin receptor,[74] thrombospondin-1 with latency-associated peptide (LAP),[75] HIV with CD4 receptor,[76] and actin with gelsolin,[77] all appear to be governed by the same principles. Furthermore, Campbell and Okada have reported that the complementary sequences of antigenic peptides may be used to map corresponding antigen binding sites in major histocompatability complex (MHC) class II molecules, which underlines the fact that binding of antigenic peptides to MHC class II molecules may involve specific sense/complementary interactions.[78] T-cell receptors have also been found to invoke sense/complementary peptide relationships for binding.[79] Even contacts between some enzymes and their substrates appear to be governed by sense/complementary peptide interactions.[80]

The potential importance of sense/complementary peptide interactions in guiding the association between peptide/protein ligands and their receptors has been reinforced by the results of several computational studies. Biro was the first to conduct a computational comparison between DNA and peptide sequences of some protein ligands and their putative receptors.[81] This revealed that sequences of ligands and putative receptors exhibit many regions of mutual complementarity that could code for putative ligand/receptor contact points. Other computational analyses of DNA and peptide sequences belonging to a wide range of protein/peptide ligands and their putative receptors (or vice versa) confirm Biro's analysis to varying extents.[24, 82–85] This has raised some obvious questions about the evolutionary origin of ligand/receptor pairs leading Blalock and Bost to impute that proteins and their receptors may have been coded for, at one time, by corresponding sense and complementary strands of DNA.[86] This now seems all the more credible in view of the evidence accumulating to show that both sense and complementary strands of DNA may be harnessed for mRNA transcription.[1, 87] Subsequently, Brentani pointed out that exon duplication followed by mutations and exon shuffling could render those corresponding sense and complementary strands of DNA independent, thereby allowing both to be expressed separately giving rise to specialised ligand/receptor pairs.[7, 88] This too is credible in the light of the discovery by Adelman et al. that mRNA coding for gonadotropin-releasing hormone in rat brains is complementary to the mRNA coding for another protein in rat cardiac muscle.[89]

One perceived drawback to the proposed strategic importance of sense/complementary peptide interactions in guiding receptor/ligand contacts is the obvious problem of "stop" codons found in complementary mRNA transcribed from complementary strand DNA. Some complementary codons are "stop" signals irrespective of whether they are read in the 5′ →3′ or 3′ →5′ direction (see Tables 1 and 3). Consequently, comple-

mentary-strand DNA might not be expected to have the same, consistent number of open reading frames (ORFs) as sense-strand DNA. Nevertheless, Goldstein and Brutlag have reported that a long ORF exists in the complementary DNA strand of the adrenocorticotropin hormone (ACTH) β-endorphin precursor (bovine POMC).[90] Also, actual numbers of "stop" signals were found to be less than half the number expected statistically in the complementary strand DNA sequence of 22 other neuropeptides. Even if this were not sufficient, reports concerning mRNA transcription from complementary strand DNA provide compelling evidence for the existence of other ORFs in the complementary strand of DNA as well.[1]

Currently, no comprehensive structural analyses, involving X-ray crystal structures of receptor/ligand complexes, have been reported to back up the apparent functional importance of sense/complementary interactions in ligand binding by receptors and their subsequent activation. Such evidence would be very important and could significantly enhance our understanding of the roles of mutually complementary peptide sequences. For instance, such a study would be able to establish the extent to which mutually complementary peptide sequences are actually in contact or in near proximity within the known structures of receptor/ligand pairs. Moreover, where they are not, alternative functional roles for these mutually complementary sequences could at least be proposed and investigated, thereby leading to an improved molecular understanding of receptor/ligand interactions and subsequent receptor activation processes.

5.3. Intramolecular sense/complementary peptide interactions and protein structure

In the same way that sense/complementary peptide interactions are being understood to play a significant role in receptor/ligand interactions, they may also have a concurrent role within protein structures.[23, 83, 91] Baranyi et al. were the first to describe experimentally intramolecular sense/complementary relationships within proteins in the form of clusters of structural motifs known as antisense homology boxes (AHBs).[92] Baranyi et al. conducted an analysis of protein databank sequence information and discovered significantly more AHBs (one per fifty residues on average) than were expected statistically. Moreover, over 64% of these AHBs were found to map to β-turn regions of proteins when three-dimensional structural information was available to make this analysis. Draper has independently reported the existence of regions of mutual complementarity within the mRNA sequence of human albumin as well.[93] Furthermore, these regions were found frequently to code for loop or β-turn regions in the protein itself. Perhaps β-turn AHBs represent strategic intrachain interaction points at an early stage in protein folding, drastically reducing the numbers of degrees of freedom in the folding polypeptide chain, to promote correct registration and assembly of secondary and tertiary structures. However, tantalising though this idea might be, early applications have not provided much support.[94]

6. Applications of Sense/Complementary Peptide Interactions

6.1. Antibodies for receptor identification and anti-autoimmunity

As part of their initial experimental work on sense/complementary peptides, Blalock and co-workers implied that the complementary peptides of peptide hormones should be able to mimic the binding-site structure of their respective hormone receptors.[27] In which case, antibodies raised against these complementary peptides should be able to target the peptide binding sites of respective hormone receptors, acting as surrogate peptide hormones capable of identifying receptors in vitro and in vivo.[27, 95] Obviously, this idea was based upon the general principle that the molecular recognition of a given receptor by its peptide hormone is governed at least in part by the interaction between receptor and hormone peptide sequences that are mutually complementary. The growing evidence for this has been discussed at length above.

Blalock and co-workers were the first to test this idea out by using antibody surrogates raised against the complementary peptide of the adrenocorticotropin hormone (ACTH) and hence targeted at the adrenocorticotropin receptor.[27] Subsequently, antibody surrogates were raised successfully to target the receptors of other peptide hormones and proteins such as γ-endorphin,[33, 60] luteinising hormone releasing hormone (LHRH),[96] angiotensin II (AII),[97] fibronectin,[98] arginine vasopressin (AVP)[99] and substance P (SP).[44] The last is particularly instructive. Polyclonal antibodies were raised against the complementary peptide of SP and were shown to bind specifically to IM-9 cells (an SP receptor positive lymphoblast cell line). This effect was inhibited in a dose-dependent manner by competition with SP peptide hormone. Subsequently, IM-9 cells were then homogenised and applied to an immunoaffinity column prepared with the same polyclonal antibodies. Following this, a bonefide SP receptor protein was found bound to the column that was eluted and shown to recognise ^{125}I-Tyr^{8}-SP. This effect was also inhibited in a dose-dependent manner by competition with unlabelled SP peptide hormone. Taken together, all the results mentioned above give a clear demonstration that antibodies raised to the complementary peptides of peptide hormones could be powerful tools for the future identification and subsequent isolation of peptide hormone receptors from appropriate cell lines.

An alternative application for antibodies raised against complementary peptides has been in the arena of autoimmunity. In autoimmune diseases, anti-self antibodies (autoantibodies) usually cause disease by targeting specific self-epitopes. For instance in myasthenia gravis (MG), an irregular antigenic response to the nicotinic acetylcholine (AChR) receptor on muscle appears to be a main pathophysiological trait of the disease.[100] An obvious therapeutic strategy is to generate anti-idiotypic antibodies specific for the idiotypes of autoantibodies. Complementary peptides may be used to generate such anti-idiotypic antibodies owing to the fact that many antibody/antigen, antibody/epitope and even idiotypic/anti-idiotypic

antibody interactions appear to be mediated by interactions between mutually complementary peptide sequences.[18, 95, 101, 102] On this basis, the complementary peptide corresponding to a given self-epitope should be reasonably equivalent to the self-epitope binding site (idiotype) of the corresponding autoantibody. Hence, antibodies raised against such a complementary peptide should be specific for the self-epitope binding site of the autoantibody and, therefore, be themselves anti-idiotypic antibodies. The best working example of this idea in practise has been in the raising of antibodies against complementary peptides corresponding to reactive AChR self-epitopes in MG. These presumably anti-idiotypic antibodies have convincingly blocked the progress of MG in a rat model of the disease.[100, 103] Furthermore, complementary "peptide vaccines" have also been designed to prevent the progress of experimental allergic neuritis (EAN).[104] Finally, encephalomyelitis has also been blocked in animal models of the disease by using a similar approach, though with more mixed results.[105, 106]

6.2. Complementary peptides as inhibitors

Complementary peptides could have all the makings of versatile inhibitors of protein/protein interactions with therapeutic applications. Taylor et al. were amongst the first to demonstrate this with respect to the inhibition of platelet adhesion.[107] Subsequently, Baranyi et al. were able to show that endothelin peptide (ET-1) could be inhibited in an in vitro smooth muscle relaxation assay by a complementary peptide whose sequence was derived from a 14 amino acid residue segment of the human endothelin A receptor.[92] Similarly, C5a anaphylatoxin was inhibited by a complementary peptide derived from a segment of the C5a receptor.[108] Even neutrophil activation may be inhibited with complementary peptides.[109] However, some of the most definitive work in this area has been that of Davids et al. who described the concept of a complementary (or antisense) peptide mini-receptor inhibitor.[26] This complementary peptide inhibitor was deliberately designed to interact with a key surface loop (or β-bulge) of interleukin-1βIL-1β) in order to sterically prevent IL-1β (and also IL-1α) from interacting with the interleukin Type 1 receptor (IL-1R), thereby inhibiting biological responses. Not only was inhibition clearly seen in vitro, but the interaction between IL-1β and the peptide was studied in detail (dissociation constant, $K_d \approx 10\ \mu M$) with appropriate controls, leaving little doubt that there was a specific interaction between peptide and the surface loop region of the protein. Subsequently, Heal et al. demonstrated the utility of this complementary peptide in an independent in vitro assay.[24]

A number of other research groups have reported the use of complementary peptides as inhibitors, including Gho and Chae who described the use of both 3′ →5′ and 5′ →3′ complementary peptides corresponding to the receptor binding site region of angiogenin.[66] They not only observed that both peptides were able to bind to angiogenin (dissociation constant, $K_d \approx 44\ nM$) but that both peptides were also able to inhibit angiogenin activity in several different in vitro assays. These receptor binding complementary peptides could be described as complementary peptide mini-effector inhibitors. Very topically, Martins et al. recently reported that prion neurotoxicity could be blocked in vitro by using a complementary peptide corresponding to the neurotoxic region of the prion protein.[110] Furthermore, they raised an antibody against this complementary peptide and were able to use this antibody to locate a cell-surface receptor for the prion protein. This suggests that prion neurotoxicity is associated with binding of the neurotoxic region to a highly specific cell-surface receptor whose binding site was mimicked by the complementary peptide. There is no doubt that the pharmaceutical potential of complementary peptide mini-receptors and similar could be great. But only time will tell if that is indeed true. Finally, there could even be a future for complementary peptides as receptor agonists under some circumstances too.[111]

6.3. Agents for affinity column purification

Initial reports on the use of complementary peptides in high-performance affinity chromatography (HPAC), or high-performance liquid affinity chromatography (HPLAC), came from Chaiken and co-workers.[8, 12, 13, 112] However Fassina and co-workers,[63, 65] have also used HPAC/HPLAC extensively for the purification of proteins including interleukin-1β (IL-1β),[62] big endothelin,[55] tumour necrosis factor-α(TNFα)[64] and interleukin-2 (IL-2).[113] Others have also followed suit.[114] The principles and operation of HPAC/HPLAC with complementary peptides are very simple. Complementary peptides specific to surface accessible regions of proteins of interest are immobilised on a column. Thereafter mixtures containing the protein may be applied to the column and eluted slowly. Owing to specific interactions between immobilised peptides and the protein of interest, the protein may be eluted in a purified state. Practical purification of proteins by this technique has the real advantage of allowing protein purification to take place with a minimum number of steps and under mild and nondenaturing conditions.

7. Summary and Outlook

There is now a sizeable body of evidence to suggest that specific interactions between sense and complementary peptides exist and may be used for a number of applications. Successful applications have been found for complementary peptides in generating anti-idiotypic antibodies in defence against autoimmunity, as probes for novel receptor proteins, as mini-receptor inhibitors of protein effectors otherwise difficult to antagonise and in high-performance affinity chromatography. A significant body of circumstantial evidence suggests that interactions between sense and complementary peptides could represent a strategic subset of the total set of molecular interactions which guide molecular recognition and binding within and between protein molecules. A global search of all genome and proteome databases for sense/complementary peptide interactions could yield important information concerning protein/protein interaction sequences, thereby giving form and function to thousands of orphan or otherwise poorly characterised genes which fill out these databases.[115] In this vein, the identification of

sense/complementary peptide relationships in and between databases could offer a useful chemo-bioinformatics tool for functional genomics. Clearly, given the complexity of protein three-dimensional structure and likely variations in sense/complementary peptide binding affinities, not all the identified relationships will be meaningful. However, simply judging by the evidence presented to date, many could be. Accordingly, software tools able to interrogate genome and proteome databases for sense/complementary peptide relationships could have a powerful role to play in the identification and validation of novel leads for drug discovery in the pharmaceutical and biotechnology industry of the postgenomic era. Cost savings to otherwise expensive combinatorial chemistry research programmes could also be possible.

The evidence to date, summarised in this review, suggests that specific interactions between sense and complementary peptides may well be mediated by M-I pairs (and other closely related amino acid pairs; Figure 2), according to the M-I pair theory. However, this theory relies on the assumption that the interacting sequences are both in an extended conformation, which is reasonable for short peptides in solution but arguably less so for interacting sequence segments in proteins where the three-dimensional organisation may be complex. Therefore, significant structural and modelling studies are required to provide firm structural and mechanistic evidence to supplement the results of functional studies that already demonstrate the potential importance of mutually complementary peptide sequences or amino acid residues, and the M-I pair theory in general, in guiding intermolecular protein/protein interactions. This should take the form of intensive structural and statistical analyses of the X-ray crystal structures of known protein/peptide and protein/protein receptor/ligand complexes, supplemented by structural studies with model systems in which sense and complementary peptides are constrained in close proximity to each other. Such studies would also have the advantage of defining the most appropriate way to account mechanistically and energetically for the specific types of interactions pertinent to each M-I pair. In addition, there is still a reasonable likelihood that intramolecular interactions between mutually complementary peptide sequence segments or individual M-I pairs within an individual polypeptide could provide a key to predicting the correct fold of proteins. This proposition certainly requires proper validation through extensive experimental and theoretical studies of protein folding but is worth investigating, in our opinion.

Hence, in conclusion, without positive results from the studies described above, it would be premature to extrapolate the M-I pair theory to the proteomic code. Nevertheless, it remains a tantalising possibility that the M-I pair theory or some derivative thereof could provide the basis for understanding and constructing this proteomic code. If so, then there would surely be some satisfaction in the knowledge that not only does the genetic code determine the amino acid sequences of proteins but, together with the complementary genetic code, also provides the means to determine their functions and even their three-dimensional structures as well. As a result, not only would we have at our disposal a potentially universal means to predict protein structures de novo, but access to a complete portfolio of molecular partners in vivo, within and between organisms, across the complete phylogenetic tree from bacteria and viruses to plants to man.

[1] a) H. B. LéJohn, L. E. Cameron, B. Yang, S. L. Rennie, *J. Biol. Chem.* **1994**, *269*, 4523 – 4531; b) B. Huelseweh, B. Kohl, H. Hentschel, R. K. H. Kinne, A. Werner, *Biochem. J.* **1998**, *332*, 483 – 489; c) N. D. Walker, N. R. McEwan, R. J. Wallace, *Biochem. Biophys. Res. Commun.* **1999**, *263*, 58 – 62; d) B. J. Van den Eynde, B. Gaugler, M. Probst-Kepper, L. Michaux, O. Devuyst, F. Lorge, P. Weynants, T. Boon, *J. Exp. Med.* **1999**, *190*, 1793 – 1799.

[2] a) R. S. Root-Bernstein, D. D. Holsworth, *J. Theor. Biol.* **1998**, *190*, 107 – 119; b) J. E. Blalock, *Cell. Mol. Life Sci.* **1999**, *55*, 513 – 518.

[3] L. B. Mekler, *Biofizika* **1969**, *14*, 581 – 584.

[4] L. B. Mekler, R. G. Idlis, VINITI Deposited Doc. 1476 – 1481, **1981**.

[5] J. E. Blalock, E. M. Smith, *Biochem. Biophys. Res. Commun.* **1984**, *121*, 203 – 207.

[6] P. Y. Chou, G. D. Fasman, *Biochemistry* **1974**, *13*, 211 – 222.

[7] R. R. Brentani, *J. Theor. Biol.* **1988**, *135*, 495 – 499.

[8] I. Chaiken, *J. Chromatogr.* **1992**, *597*, 29 – 36.

[9] R. S. Root-Bernstein, *J. Theor. Biol.* **1982**, *94*, 885 – 894.

[10] S. K. Burley, G. A. Petsko, *Adv. Protein Chem.* **1988**, *39*, 125 – 189.

[11] U. Samanta, D. Pal, P. Chakrabarti, *Proteins* **2000**, *38*, 288 – 300.

[12] Y. Shai, M. Flashner, I. M. Chaiken, *Biochemistry* **1987**, *26*, 669 – 675.

[13] Y. Shai, T. K. Brunck, I. M. Chaiken, *Biochemistry* **1989**, *28*, 8804 – 8811.

[14] G. Fassina, M. Zamai, M. Brigham-Burke, I. M. Chaiken, *Biochemistry* **1989**, *28*, 8811 – 8818.

[15] G. Fassina, P. P. Roller, A. D. Olson, S. S. Thorgeirsson, J. G. Omichinski, *J. Biol. Chem.* **1989**, *264*, 11 252 – 11 257.

[16] M. C. Menziani, M. Cocchi, P. G. De Benedetti, *J. Mol. Struct.* **1993**, *286*, 95 – 108.

[17] G. Fassina, S. S. Thorgeirson, J. G. Omichinski in *Methods in Protein Sequence Analysis* (Ed.: B. Wittman Liebold), Springer, Berlin, **1989**, pp. 431 – 438.

[18] V. Hanin, O. Déry, D. Boquet, M.-A. Sagot, C. Créminon, J.-Y. Couraud, J. Grassi, *Mol. Immunol.* **1997**, *34*, 829 – 838.

[19] G. Markus, G. L. Tritsch, R. Parthasarathy, *Arch. Biochem. Biophys.* **1989**, *272*, 433 – 439.

[20] E. G. Hutchinson, R. B. Sessions, J. M. Thornton, D. N. Woolfson, *Protein Sci.* **1998**, *7*, 2287 – 2300.

[21] J. E. Zull, S. K. Smith, *Trends Biochem. Sci.* **1990**, *15*, 257 – 261.

[22] J. Kyte, R. F. Doolittle, *J. Mol. Biol.* **1982**, *157*, 105 – 132.

[23] a) J. E. Blalock, *Trends Biotechnol.* **1990**, *8*, 140 – 144; b) J. E. Blalock, *Nat. Med. (N.Y.)* **1995**, *1*, 876 – 878.

[24] J. R. Heal, S. Bino, K. P. Ray, G. Christie, A. D. Miller, J. G. Raynes, *Mol. Immunol.* **1999**, *36*, 1141 – 1148.

[25] A. Sisto in *Peptides 1992* (Eds.: C. H. Schneider, A. N. Eberle), ESCOM Science Publishers, Amsterdam, **1993**, pp. 747 – 748.

[26] J. W. Davids, A. El-Bakri, J. Heal, G. Christie, G. W. Roberts, J. G. Raynes, A. D. Miller, *Angew. Chem.* **1997**, *109*, 999 – 1004; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 962 – 967.

[27] K. L. Bost, E. M. Smith, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 1372 – 1375.

[28] R. S. Root-Bernstein, *J. Theor. Biol.* **1983**, *100*, 99 – 106.

[29] S. Aota, T. Gojobori, F. Ishibashi, T. Maruyama, T. Ikemura, *Nucleic Acids Res.* **1988**, *16*, No.SS, r315 – 402.

[30] K. L. Bost, J. E. Blalock, *J. Mol. Recognit.* **1989**, *1*, 179 – 183.

[31] K. L. Bost, J. E. Blalock, *Methods Enzymol.* **1989**, *168*, 16 – 28.

[32] D. S. Jones, *J. Chem. Soc. Perkin Trans. 1* **1972**, 1407 – 1415.

[33] D. J. J. Carr, K. L. Bost, J. E. Blalock, *J. Neuroimmunol.* **1986**, *12*, 329 – 337.

[34] J. E. Blalock, K. L. Bost, *Biochem. J.* **1986**, *234*, 679 – 683.

[35] R. L. Soffer, S. Bandyopadhyay, E. Rosenberg, P. Hoeprich, A. Teitelbaum, T. Brunck, C. B. Colby, C. Gloff, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 9219 – 9222.

[36] D. D. Holsworth, J. S. Kiely, R. S. Root-Bernstein, R. W. Overhiser, *Pept. Res.* **1994**, *7*, 185 – 193.

[37] H. M. Johnson, B. A. Torres, *J. Immunol.* **1988**, *141*, 2420 – 2423.

[38] H. M. Johnson, B. A. Torres, *Immunomethods* **1994**, *5*, 167 – 171.

[39] T. K. Gartner, R. Loudon, D. B. Taylor, *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1446 – 1452.
[40] T. K. Gartner, D. B. Taylor, *Proc. Soc. Exp. Biol. Med.* **1991**, *198*, 649 – 655.
[41] D. A. Weigent, B. L. Clarke, J. E. Blalock, *Immunomethods* **1994**, *5*, 91 – 97.
[42] R. S. Root-Bernstein, F. C. Westall, *Brain Res. Bull.* **1986**, *17*, 519 – 528.
[43] F. A. Al-Obeidi, V. J. Hruby, S. D. Sharma, M. E. Hadley, A. M. Castrucci in *Peptides, Chemistry, Structure and Biology* (Eds.: J. E. River, G. R. Marshall), **1990**, pp. 530 – 532.
[44] D. W. Pascual, J. E. Blalock, K. L. Bost, *J. Immunol.* **1989**, *143*, 3697 – 3702.
[45] J. L. Bret-Dibat, D. Zouaoui, O. Déry, F. Zerari, J. Grassi, S. Maillet, M. Conrath, J. Y. Couraud, *J. Neurochem.* **1994**, *63*, 333 – 343.
[46] D. Borovsky, C. A. Powell, J. K. Nayar, J. E. Blalock, T. K. Hayes, *FASEB J.* **1994**, *8*, 350 – 355.
[47] L. Sautebin, L. Rombolà, M. Di Rosa, G. Caliendo, E. Perissutti, P. Grieco, B. Severino, V. Santagada, *Eur. J. Med. Chem.* **2000**, *35*, 727 – 732.
[48] A. N. Eberle, M. Huber, *J. Recept. Res.* **1991**, *11*, 13 – 43.
[49] E. S. Najem, A. Corigliano-Murphy, J. A. Ferretti, *FEBS Lett.* **1989**, *250*, 405 – 410.
[50] G. Guillemette, G. Boulay, S. Gagnon, R. Bosse, E. Escher, *Biochem. J.* **1989**, *261*, 309.
[51] M. De Gasparo, S. Whitebread, K. Einsle, C. Heusser, *Biochem. J.* **1989**, *261*, 310 – 311.
[52] H. L. Eaton, R. E. Austin, S. W. Fesik, S. F. Martin, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9767 – 9769.
[53] U. B. Rasmussen, R.-D. Hesch, *Biochem. Biophys. Res. Commun.* **1987**, *149*, 930 – 938.
[54] J. Beattie, D. J. Flint, *Biochem. J.* **1992**, *283*, 473 – 478.
[55] G. Fassina, R. Consonni, L. Zetta, G. Cassani, *Int. J. Pept. Protein Res.* **1992**, *39*, 540 – 548.
[56] R. S. Root-Bernstein, F. C. Westall, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 4339 – 4342.
[57] J. A. Loo, D. D. Holsworth, R. S. Root-Bernstein, *Biol. Mass Spectrom.* **1994**, *23*, 6 – 12.
[58] K. P. Madhusudanan, S. B. Katti, W. Haq, P. K. Misra, *J. Mass. Spectrom.* **2000**, *35*, 237 – 241.
[59] J. Dillon, W. T. Woods, V. Guarcello, R. D. LeBoeuf, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 9726 – 9729.
[60] S. A. Martin-Moe, R. Lehr, M. D. Cauley, G. R. Moe, *Pept. Res.* **1995**, *8*, 70 – 76.
[61] E. V. Curto, N. R. Krishna, *Immunomethods* **1994**, *5*, 98 – 106.
[62] G. Fassina, G. Cassani, *Biochem. J.* **1992**, *282*, 773 – 779.
[63] G. Fassina, A. Corti, G. Cassani, *Int. J. Pept. Protein Res.* **1992**, *39*, 549 – 556.
[64] G. Fassina, G. Cassani, A. Corti, *Arch. Biochem. Biophys.* **1992**, *296*, 137 – 143.
[65] G. Fassina, *J. Chromatogr.* **1992**, *591*, 99 – 106.
[66] Y. S. Gho, C.-B. Chae, *J. Biol. Chem.* **1997**, *272*, 24 294 – 24 299.
[67] K. L. Bost, E. M. Smith, J. E. Blalock, *Biochem. Biophys. Res. Commun.* **1985**, *128*, 1373 – 1380.
[68] J. Ghiso, E. Saball, J. Leoni, A. Rostagno, B. Frangione, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 1288 – 1291.
[69] N. Ruiz-Opazo, K. Akimoto, V. L. M. Herrera, *Nat. Med. (N.Y.)* **1995**, *1*, 1074 – 1081.
[70] J. W. Slootstra, E. W. Roubos, *Biochem. Biophys. Res. Commun.* **1991**, *179*, 266 – 271.
[71] A. J. Sytkowski, K. A. Donahue, *J. Biol. Chem.* **1987**, *262*, 1161 – 1165.
[72] a) J. F. Bazan, *Biochem. Biophys. Res. Commun.* **1989**, *164*, 788 – 795; b) A. D. D'Andrea, H. F. Lodish, G. G. Wong, *Cell* **1989**, *57*, 277 – 285.
[73] J. Slootstra, E. Roubos, *Trends Biotechnol.* **1990**, *8*, 279 – 281.
[74] A. Wijkhuisen, M.-A. Sagot, Y. Frobert, C. Créminon, J. Grassi, D. Boquet, J.-Y. Couraud, *FEBS Lett.* **1999**, *447*, 155 – 159.
[75] S. M. Ribeiro, M. Poczatek, S. Schultz-Cherry, M. Villain, J. E. Murphy-Ullrich, *J. Biol. Chem.* **1999**, *274*, 13 586 – 13 593.
[76] R. Brown, C. Meldrum, S. Cousins, *Med. Hypotheses* **1992**, *38*, 322 – 324.
[77] R. J. Sheedy, F. M. Clarke in *Results and Problems in Cell Differentiation, Vol. 32: Molecular Interactions of Actin* (Ed.: C. dos Remedios), Springer, Heidelberg, **2001**, pp. 155 – 164.
[78] W. Campbell, H. Okada, *Biochem. Biophys. Res. Commun.* **1991**, *175*, 207 – 214.
[79] C. C. Maier, R. D. LeBoeuf, S. R. Zhou, J. N. Whitaker, J. E. Blalock, *FASEB J.* **1991**, *5*, A1383.
[80] S. J. De Souza, R. Brentani, *J. Biol. Chem.* **1992**, *267*, 13 763 – 13 767.
[81] J. Biro, *Med. Hypotheses* **1981**, *7*, 981 – 993.
[82] T. Kunisawa, J. Otsuka, *Protein Sequences Data Anal.* **1987**, *1*, 117 – 121.
[83] J. E. Zull, R. C. Taylor, G. S. Michaels, N. B. Rushforth, *Nucleic Acids Res.* **1994**, *22*, 3373 – 3380.
[84] G. Fassina, M. Melli, *Immunomethods* **1994**, *5*, 114 – 120.
[85] N. Stambuk, *Period. Biol.* **1999**, *101*, 355 – 361.
[86] J. E. Blalock, K. L. Bost, *Recent Prog. Horm. Res.* **1988**, *44*, 199 – 222.
[87] C. Vanhee-Brossollet, C. Vaquero, *Gene* **1998**, *211*, 1 – 9.
[88] a) R. R. Brentani, *J. Mol. Evol.* **1990**, *31*, 239 – 243; b) R. R. Brentani, *Trends Biochem. Sci.* **1990**, *15*, 463.
[89] J. P. Adelman, C. T. Bond, J. Douglass, E. Herbert, *Science* **1987**, *235*, 1514 – 1517.
[90] A. Goldstein, D. L. Brutlag, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 42 – 45.
[91] J. E. Blalock, *Nat. Med. (N.Y.)* **1995**, *1*, 1223.
[92] L. Baranyi, W. Campbell, K. Ohshima, S. Fujimoto, M. Boros, H. Okada, *Nat. Med. (N.Y.)* **1995**, *1*, 894 – 901.
[93] K. G. Draper, *Biochem. Biophys. Res. Comm.* **1989**, *163*, 466 – 470.
[94] A. Kierzek, D. Plochocka, P. Zielenkiewicz, *Nat. Med. (N.Y.)* **1995**, *1*, 1222 – 1223.
[95] L. R. Smith, K. L. Bost, J. E. Blalock, *J. Immunol.* **1987**, *138*, 7 – 9.
[96] J. J. Mulchahey, J. D. Neill, L. D. Dion, K. L. Bost, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 9714 – 9718.
[97] T. S. Elton, L. D. Dion, K. L. Bost, S. Oparil, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 2518 – 2522.
[98] R. R. Brentani, S. F. Ribeiro, P. Potocnjak, R. Pasqualini, J. D. Lopes, C. R. Nakaie, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 364 – 367.
[99] a) L. G. Abood, G. J. Michael, L. Xin, K. M. Knigge, *J. Recept. Res.* **1989**, *9*, 19 – 25; b) B. H. Swords, J. E. Blalock, K. H. Berecek, *Hypertension* **1989**, *14*, 352; c) B. H. Swords, D. J. J. Carr, J. E. Blalock, K. H. Berecek, *Neuroendocrinology* **1990**, *51*, 487 – 492.
[100] S. Araga, R. D. LeBoeuf, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8747 – 8751.
[101] C.-Y. Kang, T. K. Brunck, T. Kieber-Emmons, J. E. Blalock, H. Kohler, *Science* **1988**, *240*, 1034 – 1036.
[102] a) C. C. Maier, H. N. B. Moseley, S. R. Zhou, J. N. Whitaker, J. E. Blalock, *Immunomethods* **1994**, *5*, 107 – 113; b) D. Boquet, O. Déry, Y. Frobert, J. Grassi, J. Y. Couraud, *Mol. Immunol.* **1995**, *32*, 303 – 308; c) M. Villa, J.-M. Durand-Gorde, P. Carayon, J. Ruf, *J. Autoimmunity* **1996**, *9*, 653 – 660.
[103] a) S. Araga, F. S. Galin, M. Kishimoto, A. Adachi, J. E. Blalock, *J. Immunol.* **1996**, *157*, 386 – 392; b) L. Xu, S. Araga, M. Villain, F. S. Galin, J. E. Blalock, *FASEB J.* **1999**, *13*, A1123.
[104] S. Araga, M. Kishimoto, S. Doi, K. Nakashima, *J. Immunol.* **1999**, *163*, 476 – 482.
[105] S.-R. Zhou, J. N. Whitaker, *J. Immunol.* **1993**, *150*, 1629 – 1642.
[106] C. J. Xian, R. D. Simmons, D. O. Willenborg, A. A. Vandenbark, G. A. Hashim, P. R. Carnegie, *J. Neurosci. Res.* **1995**, *41*, 620 – 627.
[107] D. B. Taylor, D. L. Amrani, N. E. Kirschbaum, D. A. Walz, T. K. Gartner, *Thromb. Haemostasis* **1991**, *65*, 1149.
[108] L. Baranyi, W. Campbell, H. Okada, *J. Immunol.* **1996**, *157*, 4591 – 4601.
[109] R. R. Pfister, J. L. Haddox, J. E. Blalock, C. I. Sommers, L. Coplan, M. Villain, *Cornea* **2000**, *19*, 384 – 389.
[110] V. R. Martins, E. Graner, J. Garcia-Abreu, S. J. de Souza, A. F. Mercadante, S. S. Veiga, S. M. Zanata, V. M. Neto, R. R. Brentani, *Nat. Med. (N.Y.)* **1997**, *3*, 1376 – 1382.
[111] B. L. Clarke, J. E. Blalock, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9708 – 9711.
[112] F. X. Lu, N. Aiyar, I. Chaiken, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 3642 – 3646.
[113] G. Fassina, G. Cassani, P. Gnocchi, M. C. Fornasiero, A. M. Isetta, *Arch. Biochem. Biophys.* **1995**, *318*, 37 – 45.
[114] L. Scapol, P. Rappuoli, G. C. Viscomi, *J. Chromatogr.* **1992**, *600*, 235 – 242.
[115] H. Kohler, J. E. Blalock, *Nat. Biotechnol.* **1998**, *16*, 601.
[116] G. J. Moore, R. C. Ganter, K. J. Franklin, *Biochem. Biophys. Res. Commun.* **1989**, *160*, 1387 – 1391.
[117] K. M. Knigge, D. T. Piekut, D. Berlove, *Neurosci. Lett.* **1988**, *86*, 269 – 271.
[118] N. A. Shahabi, K. L. Bost, T. C. Madhok, B. M. Sharp, *J. Pharmacol. Exp. Ther.* **1992**, *263*, 876 – 88.
[119] G. M. Nagy, L. S. Frawley in *73rd Annual Endocrine Society Meeting* **1991**, p. 209 [Abs. 716].
[120] X. Wu, N. T. Richards, E. J. Johns, T. Kohsaka, A. Nakamura, H. Okada, *Br. J. Pharmacol.* **1997**, *122*, 316 – 320.
[121] D. J. J. Carr, J. E. Blalock, K. L. Bost, *Immunol. Lett.* **1989**, *20*, 181 – 186.

[122] P. K. Misra, W. Haq, S. B. Katti, K. B. Mathur, R. Raghubir, G. K. Patnaik, B. N. Dhawan, *Pharm. Res.* **1993**, *10*, 660 – 661.
[123] N. Stambuk, N. Kopjar, K. Sentija, V. Garaj-Vrhovac, D. Vikic-Topic, R. M. D. Marina, V. Brinar, M. Trobojevic-Cepe, N. Zarkovic, B. Curkovic, D. Babic-Naglic, M. Hadzija, N. Zurak, Z. Brzovic, R. Martinic, V. Stambuk, P. Konjevoda, N. Ugrinovic, I. Pavlic-Renar, Z. Bidin, B. Pokric, *Croat. Chem. Acta* **1998**, *71*, 591 – 605.
[124] R. Pasqualini, D. F. Chamone, R. R. Brentani, *J. Biol. Chem.* **1989**, *264*, 14 566 – 14 570.
[125] J. E. McGuigan, M. Campbell-Thompson, *Gastroenterology* **1992**, *103*, 749 – 758.
[126] C. E. Grosvenor, K. Balint, US 4833166, **1987**.
[127] M. Imai, N. Okada, H. Okada, *Microbiol. Immunol.* **2000**, *44*, 205 – 212.
[128] J. E. Blalock, J. N. Whitaker, E. N. Benveniste, K. L. Bost, *Methods Enzymol.* **1989**, *178*, 63 – 74.
[129] V. P. Knutson, *J. Biol. Chem.* **1988**, *263*, 14 146 – 14 151.
[130] J. M. Derrick, D. B. Taylor, R. G. Loudon, T. K. Gartner, *Biochem. J.* **1997**, *325*, 309 – 313.
[131] H. M. Johnson, M. P. Langford, B. Lakhchaura, T.-S. Chan, G. J. Stanton, *J. Immunol.* **1982**, *129*, 2357 – 2359.
[132] D. A. Weigent, P. D. Hoeprich, K. L. Bost, T. K. Brunck, W. E. Reiher III, J. E. Blalock, *Biochem. Biophys. Res. Commun.* **1986**, *139*, 367 – 374.
[133] V. Castronovo, G. Taraboletti, M. E. Sobel, *Cancer Res.* **1991**, *51*, 5672 – 5678.
[134] A. Bajpai, K. P. Hooper, K. E. Ebner, *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1312 – 1317.
[135] D. J. J. Carr, B. DeCosta, A. E. Jacobson, K. L. Bost, K. C. Rice, J. E. Blalock, *FEBS Lett.* **1987**, *224*, 272 – 276.
[136] M. Campbell-Thompson, J. E. McGuigan, *Am. J. Med. Sci.* **1993**, *305*, 365 – 373.
[137] J. M. Kelly, D. Trinder, P. A. Phillips, D. J. Casley, B. Kemp, V. Mooser, C. I. Johnston, *Peptides (N.Y.)* **1990**, *11*, 857 – 862.

Received: September 12, 2000
Revised version: July 9, 2001 [A 132]