Role of complementary proteins in autoimmunity: an old idea re-emerges with new twists

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It has been suggested that complementary proteins are involved in autoimmunity through a network involving idiotype–anti-idiotype reactions termed 'autoantigen complementarity'. We propose that complementary proteins, which occur naturally or result from cellular dysfunction, might be more common than recognized currently. This implies that the role of complementary proteins in autoimmunity merits increasing investigation. The concept of complementary proteins is reviewed here and, also, new ideas are presented that underscore the role of open-reading frames in frame −1 of recognized genes in the production of complementary proteins (frame −1 is the reverse complement sequence of a gene that uses the antisense of the codons of frame +1). Furthermore, a novel role for palindromic sequences in autoimmunity and a new model explaining how abzymes and autoantigen complementarity might be related are proposed.

Introduction

Despite advances in understanding the targets of autoimmune destruction, the causes of autoimmunity remain unclear. Mounting evidence suggests that complementary proteins (see Glossary) might have a role in the induction of autoimmunity through a complex network of idiotypic–anti-idiotypic antibodies [1–4] (reviewed in Ref. [5]). This idea was initially promulgated nearly two decades ago [6] and further substantiated by the discovery [7,8] that a protein, termed cPR-3 (c, complementary), encoded by the antisense strand of the human *proteinase-3* gene, is able to initiate an autoimmune response by first eliciting the formation of antibodies against itself. These antibodies, in turn, provoke the formation of antibodies against themselves and these are able to recognize and bind proteinase-3, which results in an autoimmune response. This process is termed 'autoantigen complementarity' [8] and results in the formation of an idiotype–anti-idiotype network (Box 1).

What are complementary proteins?

In the context of this discussion, a complementary protein is one that is encoded by the antisense strand of a gene. In particular, we focus on complementary proteins derived from the translation of frame −1 (Figure 1a). If two proteins have been proved experimentally to be encoded by the sense and anti-sense strands of a gene, respectively, then it is arbitrary to define which is sense and which is antisense. However, the protein product of one strand is

Box 1. Scheme for the generation of an autoimmune reaction according to the autoantigen complementarity theory

Transcription and translation of the sense and antisense strand of a gene give rise to complementary proteins, which have epitopes (Figure I**a,b**) that are structurally or sequence related in a complementary way. Complementary proteins might interact physically. The protein encoded by the sense strand is involved in normal cellular function. The antisense protein might also be a naturally occurring protein but could also be one that is induced by cellular dysfunction from a normally silent ORF in the antisense gene. An antibody **(c)** can be generated against the antisense epitope **(b)** that, in turn, can elicit the formation of an anti-idiotypic antibody **(d)** against itself. This anti-idiotype antibody can then interact with the epitope **(a)** resulting in an autoimmune reaction. We propose that, if the sense protein **(a)** has catalytic function, then the antibody **(c)** might also have catalytic activity and, therefore, would be considered an abzyme. An abzyme thus becomes a potential intermediate in autoantigen complementarity.

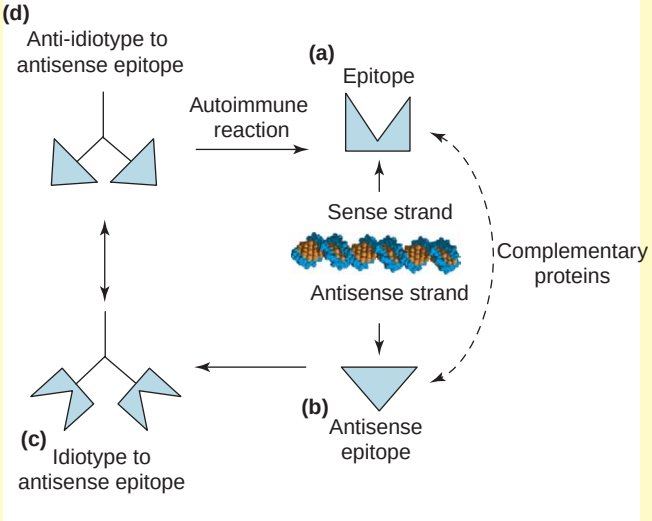

Figure I.

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Glossary

Abzyme: an antibody that has catalytic activity.
Autoantigen complementarity: a theory postulating that a protein or short peptide encoded by the antisense strand of a gene can initiate a chain of events leading to the formation of an antibody against the protein encoded by the sense strand of the gene, thus evoking an autoimmune attack (Box 1).
Complementary protein: this has several meanings, depending on the biological context. In the theory of molecular recognition and autoantigen complementarity, it is a protein encoded by the antisense strand of a gene that has structural and/or sequence complementarity with the protein encoded by the sense strand. We postulate that the majority of complementary proteins arise from frame −1 of the antisense strand.
Hydropathy: a measure of the tendency of an amino acid to enter a non-polar (hydrophobic) environment.
Molecular recognition: a theory postulating that two proteins derived from the sense (frame +1) and anti-sense strands (frame −1), respectively, of a gene exhibit complementary structures or amino acid sequences that promote their mutual recognition and interaction. Although several plausible models have been proposed, including mirror image hydropathy, a detailed understanding of the phenomenon at the molecular level awaits further investigation. There are also several additional explanations that do not rely on the concept of interacting proteins derived specifically from frame +1 and frame −1 of a gene.

generally recognized first, either experimentally or by bioinformatic prediction, and is considered, by convention, to be the product of the sense strand. A subsequently validated or predicted protein, derived from the opposite strand, then becomes the antisense protein.

Idiotypic networks generated by complementary proteins

There is extensive evidence that complementary proteins and peptides induce idiotype–anti-idiotype networks. In some instances, these networks have the ability to protect from disease. For example, in myasthenia gravis, high affinity antibodies are produced to the acetylcholine receptor (AChR), which is driven by self-responsive T cells. Immunizing animals with a complementary peptide to AChR induced antibodies that block T-cell activity and inhibit the binding of anti-AChR antibodies, thus decreasing disease [1,9]. However, complementary peptides also induce idiotype–anti-idiotype networks [6] and the presence of these networks can be shown in autoimmunity [2]. Patients with Sjögren's syndrome and systemic lupus erythematosus often contain idiotype–anti-idiotype antibodies to La/SSB (lupus/Sjögren's syndrome autoantigen B), an autoantigen in both of these diseases. Both the antigenic epitopes from this protein and their complementary peptides can induce idiotype–anti-idiotype antibodies [3] and strong T-cell reactivity [4] to both peptides in non-autoimmune mice. Therefore, these idiotypic–anti-idiotypic networks could contribute to the development of autoimmunity if the antisense products are expressed ectopically.

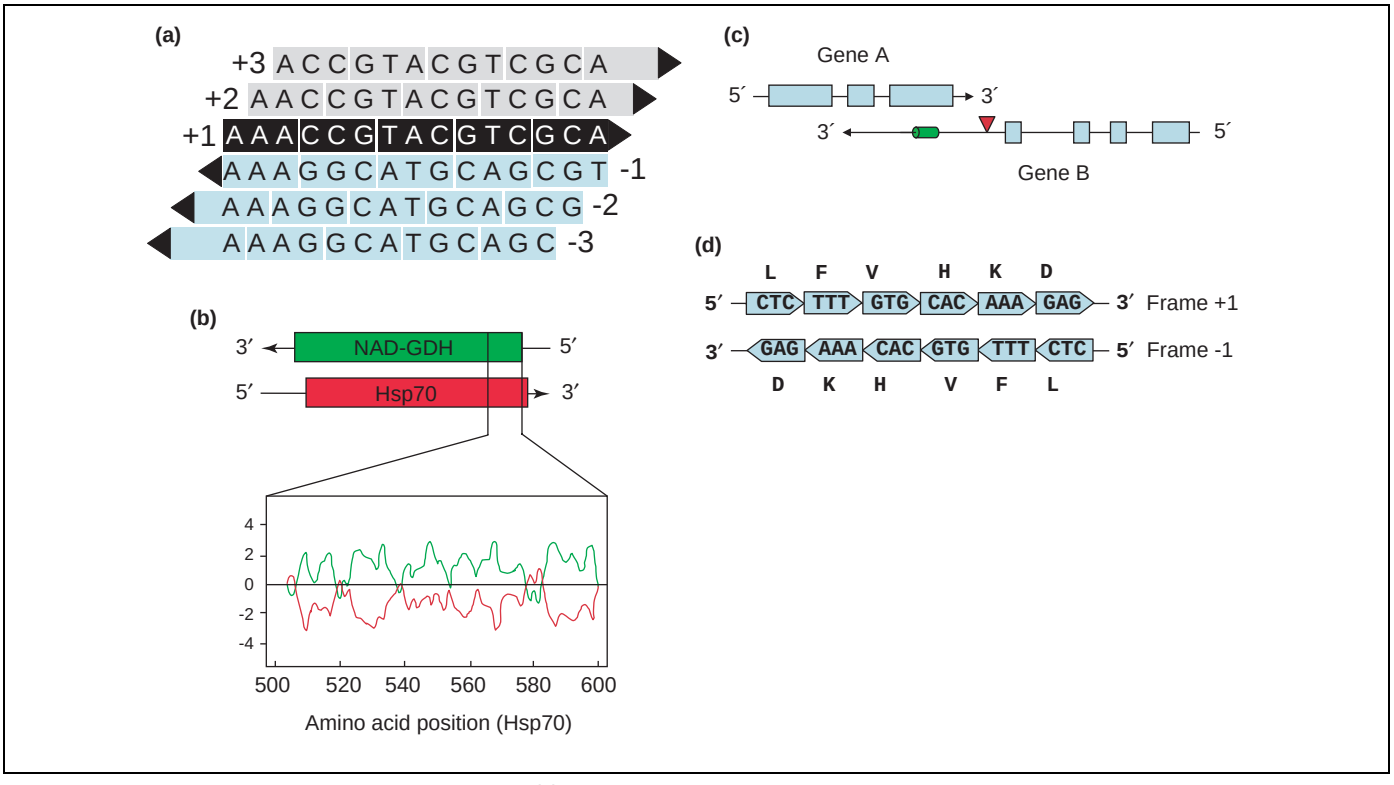

Figure 1. Concepts involved in complementary protein synthesis. **(a)** The six potential reading frames of a gene. Each box represents a codon. The arrowheads indicate the direction of translation. The black boxes are codons of the known gene, defined as frame +1. There are two additional reading frames (grey boxes) in the same direction as the known gene (frames +2 and +3) and three in the antisense direction (−1, −2 and −3) (blue boxes). The second position of frame +1 is superimposed on the second codon position of frame −1. **(b)** Hydropathy plot of part of the amino acid sequence (coded in the sense strand) of NAD-glutamine dehydrogenase (NAD-GDH) of the water mold *Achlya*, compared to the sequence of the heat shock protein 70 (Hsp70) chaperone that is coded by frame −1 in the opposing antisense strand. The plots of the hydropathy index of the two proteins are almost mirror images (modified, with permission, Ref. [26]). Arrows indicate the orientation of the genes. Both proteins are shown in their N-terminal to C-terminal orientation. **(c)** Hypothetical model suggesting how read-through of a gene (Gene B) could lead to the generation of a new exon (green tube), antisense to a known exon (Gene A). Blue boxes represent exons; the solid lines represent the transcriptional units of the two genes and the arrowheads indicate direction and termination of transcription. The red triangle marks the normal termination of transcription for gene B that, in the model, has mutated to enable transcriptional read-through to a new downstream termination site. **(d)** Illustration of how a palindromic DNA sequence can give rise to the same amino acid sequence encoded by both strands of the DNA. Blue boxes are codons and the letters are amino acids.

This mechanism has been shown to be involved in one case of autoimmune vasculitis. Pendergraft *et al.* [7] demonstrated the production of antibodies specific for a protein called cPR-3, produced from an antisense transcript derived from the *proteinase-3* gene. Proteinase-3 is the normal target of the autoantibodies but both antisense transcripts and antibodies to the antisense product could be detected in patients. In addition, mice immunized with cPR-3 produce both cPR-3- and proteinase-3-specific antibodies, thus demonstrating the presence of an idiotype–anti-idiotype network. This is the only known example of a complementary protein involved in autoimmunity but it leads to the hypothesis that this novel mechanism might be relevant to other autoimmune diseases.

What is the molecular basis for molecular complementarity?

A key assertion of the autoantigen complementarity theory is that proteins encoded by the antisense strand can exhibit structural or sequence-related symmetry that is complementary to those encoded by the sense strand. Three main theories, proposed by Root-Bernstein and Holsworth [10], Siemion *et al.* [11] and Smith *et al.* [6], have been put forward to explain this proposed symmetry [5,10–16]. All three hypotheses use ideas regarding amino acid interactions developed by Meckler [17] and all three have been exploited to design complementary peptides that can be used to block protein–ligand interactions [15,18–21] or to design vaccines [5,9].

The theories proposed by Root-Bernstein and Holsworth and Siemion *et al.* rely on concepts based on stereochemical complementarity of amino acids and anti-amino acids. Anti-amino acids are conceptually translated by reading the antisense strand in the 3′ to 5′ direction. Their order can be encoded in a synthetic nucleic acid that is translated in the standard 5′ to 3′ direction. This strategy has met with some success for the design of artificial complementary peptides [18] but is not relevant when considering naturally occurring complementary peptides because there is no known mechanism for translating mRNA in the 3′ to 5′ direction.

The third explanation for protein complementarity involves the concept of molecular recognition, proposed by Blalock and co-workers [5,6,12], with contributions by others (e.g. [22–26]). A special coding property in the relationship between frame +1 and −1 of a gene was noted, namely: their respective codons, although antisense to each other, are in phase (Figure 1a). This superimposes the second codon position of both frames. This codon position gives rise to the phenomenon of reverse or mirror-image hydropathy because T(U) in the second codon position specifies only the hydrophobic amino acids Phe, Leu, Ile, Met and Val, whereas A in the second position on the opposite strand encodes principally hydrophilic amino acids, Tyr, His, Gln, Asn, Lys, Asp and Glu. This can result in proteins that exhibit substantial mirror-image hydropathy [26] (Figure 1b) and is the foundation for postulating complementary structural profiles that might not only promote protein–protein interaction but might also be the basis for the occurrence of complementary epitopes.

This idea provokes additional questions. Is the occurrence of an open-reading frame (ORF) in frame −1 frequent or is it of minor biological significance? Are the size and quality of the coding information in frame −1 such that they could be expected to encode stable proteins? This is necessary if the protein is to assume a stable tertiary structure that can interact with its partner from frame +1 and can survive long enough to elicit an antibody response. We argue later that the answer to these questions is a qualified 'yes'.

But beyond these considerations is the issue of whether frame −1 is translated with sufficient frequency to make autoantigen complementarity a significant mechanism in the development of autoimmune diseases. An increasing number of examples of naturally occurring proteins derived from frame −1 are found that might initiate the events leading to autoantigen complementarity. However, we argue that, in addition to these, there could be many more instances of proteins or peptides encoding from frame −1 that occur as the result of cellular dysfunction.

Frame −1 has properties that make it likely to encode complementary proteins

We suggest that frame −1 has three additional properties that could link it to the appearance of complementary proteins:

(i) It is the frame most likely to have a stretch of nucleotides with an ORF of sufficient length to encode a peptide long enough to permit stable folding and/or to form epitopes. The reasons why this frame is favored for the development of ORFs are discussed in Ref. [27].

(ii) It is the frame that has the most similarity to frame +1 with respect to codon usage [27]. Therefore, it would be translated efficiently if coupled with proper regulatory sequences, such as ribosome entry sites.

(iii) If translated, frame −1 would encode proteins with a fairly normal composition of amino acids [27]. Therefore, these proteins would have the potential to form biologically significant folds that would reduce their tendency to aggregate and enhance their survival from cellular surveillance and proteolytic degradation mechanisms.

These three important properties are not exhibited by any of the other four alternate reading frames (not counting frame +1) [27].

Origin of autoantigenic complementary proteins

Examples of naturally occurring, complementary proteins are known [28–30], including those thought to be interacting partners [26,31]. However, a natural complementary protein should have been screened by tolerance mechanisms and so would not be expected to evoke an autoimmune response under normal circumstances.

It is assumed that the majority of ORFs in frame −1 of known genes are not translated because they are not connected to transcription and translation regulatory sequences. However, several types of genetic error, such as recombination, transposition, retrotransposition,

capture of splice sites, read-through from upstream transcriptional units and translation of regulatory antisense RNA, could provoke the transcription and/or translation of normally 'silent' antisense ORFs, resulting in the creation of new complementary proteins that would not have been screened by tolerance mechanisms.

Because we argue that substantial ORFs occur abundantly in frame −1 and that this frame is the most likely to encode stable proteins, we suggest that examples of novel complementary epitopes should be searched for within this frame. A probable potential source of such epitopes would be through transcriptional read-through of antisense ORFs from upstream genes (Figure 1c). Another important source of epitopes could be the translation of normally silent ORFs embedded in antisense RNA. Given the large number of predicted antisense RNA genes in the human genome, some of which occur antisense to known coding regions [32–34], it will be important to determine whether any of these can give rise to antisense peptides that could act as epitopes. Alternatively, novel complementary epitopes could come from the translation of antisense genes introduced by infectious organisms, which has been reviewed previously [8,35].

Is there a link between antisense RNA and autoimmunity?

There are several striking examples potentially linking antisense transcription, and associated complementary protein synthesis, to autoimmunity. There are several known examples of antisense transcription in the brain [36–40] and there are several autoimmune diseases of this tissue. Antisense transcription is also associated with inherited cases of neuropathy [41,42], suggesting that antisense transcripts might be linked to neuronal dysfunction. Ribonucleoproteins, such as that encoded by the *La/SSB* gene, are common targets in autoimmune disease as well (reviewed in Ref. [43]). Recently, a nucleolar protein called ASE-1, encoded by an antisense transcript from the *ERCC-1* (excision repair gene that complements repair mutant CHO cells) DNA repair enzyme gene, was discovered using human autoimmune serum [44]. Another ribonucleoprotein, RBM8A, is encoded by a gene antisense to the *type II gonadotropin-releasing hormone receptor* gene [45]. It is not known whether antisense transcripts arise from the other genes that encode ribonucleoprotein components but it is worth exploring whether or not these genes can encode antisense transcripts and/or complementary proteins. It would be intriguing if both antisense transcripts and complementary protein expression were involved in the complex series of events that leads to the development of autoimmune disease.

Can the creation of palindromic sequences in coding sequences elicit autoimmunity?

As an alternative hypothesis to account for autoantibodies, we suggest the possibility that palindromic DNA sequences (inverted repeats) might occasionally be transcribed and translated from the antisense strand of a gene. In this case, the proteins derived from the sense and antisense strands will share a segment of identical, or near identical, amino acids (Figure 1d), embedded in different amino acid sequences encoded by codons lying outside the palindrome. Thus, antibodies might arise against the novel antisense protein that could then recognize epitopes in the protein translated from frame +1, resulting in an autoimmune response. Where could such palindromic sequences come from?

Mobile elements, such as transposons, insertion sequences, Lines and Sines, constitute a large proportion of the human genome and often contain palindromes at their termini. Because there is evidence for the widespread remains of such elements within human coding sequences [46], such molecular fossils represent an abundant source of palindromic sequences In addition, events that provoke the movement and integration of mobile elements within the genome, such as chromosome breakage, could provide a novel source of palindromic sequences within genes.

Palindromic sequences can also be created by genetic errors that are associated with diseases. These errors increase the rate of palindrome formation by mechanisms that are not well understood. In a recent study, palindromes were found at high frequency and in a non-random distribution in certain cancer cells [47], and it would be interesting to determine if any of the known examples of autoimmunity associated with cancer result from the creation of antibodies to epitopes arising from palindromic sequences translated from the antisense strands of genes.

Is there a relationship between complementary proteins and abzymes that could lead to a novel mechanism of autoimmunity?

Abzymes are antibodies with catalytic function [48]. There are >100 known different abzymes, some of which were generated artificially by injecting quasi-stable enzymatic reaction intermediates into animals as the corresponding antigen. However, some abzymes arise naturally and their association with autoimmune states has been well established ([49] and references therein). Some abzymes might contribute directly to autoimmune disease, such as in the destruction of the myelin sheath in patients with multiple sclerosis [50]. However, there is another possible mechanism by which abzymes could contribute to autoimmune diseases. We propose that abzymes can arise as antibodies to antisense epitopes (Box 1). If an antisense protein arises from a gene encoding an enzyme and it contains an epitope complementary to the active site of the enzyme, then, according to the molecular recognition theory, an antibody to that epitope could exhibit similar catalytic activity. The antibody would be an abzyme and it could induce anti-idiotypic antibodies that recognize the enzyme and destroy it. If this happens, an abzyme would represent an intermediate in the chain of events that leads to autoimmunity, according to the hypothesis of autoantigen complementarity. Of course, abzymes to intracellular components would have to enter the cell to cause damage and it is unclear how often this would actually occur or if these antibodies could contribute to disease.

There are several testable predictions of this theory. In cases of autoimmunity associated with abzymes, the following should occur: (i) the synthesis of an antisense epitope (Box 1, Figure Ib) to a gene that has the catalytic

Box 2. Outstanding issues

- Suspected autoantigen genes in autoimmune and normal tissues should be investigated for the presence of antisense transcription
- The role of frame −1 in generating antisense proteins should be further evaluated
- Suspected autoantigen genes should be investigated to determine if they encode complementary proteins
- Complementary protein expression should be verified *in vivo* in cases of autoimmunity versus normal tissues
- Bioinformatics should be used to determine the coding potential of antisense genes in known autoantigens
- Pathogenic microbial genomes should be examined for possible antisense coding potential that might elicit autoimmune responses
- The proposed relationship between abzymes and autoantigen complementarity should be investigated
- A deeper understanding of the chemical basis of molecular recognition is required to understand more profoundly the events involved in autoantigen complementarity

function proposed for the abzyme, (ii) detectable reactions between the proposed abzyme (Box 1, Figure Ic) and the antisense epitope and between the abzyme and the postulated anti-idiotypic antibody (Box 1, Figure Id) and also, (iii) a reaction between the anti-idiotype and the natural enzyme (Box 1, Figure Ia). It is not a requirement for this model that sense and antisense transcription and translation occur concurrently in the same cell but, in the instances that they do, an additional prediction can be made, namely (iv) that the appearance of an abzyme should be accompanied by a concomitant decrease in activity of the corresponding cellular enzyme. If this theory is correct, it suggests that abzymes might be especially prominent as a cause of autoimmunity because they do not only directly contribute by destroying self-tissue components but they also indirectly contribute by eliciting anti-idiotype antibodies that can attack self.

Concluding comments

The literature reviewed here suggests that there might be a link between antisense transcription, complementary proteins and autoimmunity, and we propose that the possible association warrants further examination. This, and other outstanding issues, is outlined in Box 2. Antisense transcription and/or the production of complementary proteins might prove to have an important role in many types of human disease, including cancer.

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