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Structure and Expression of Polypeptides Encoded in the Mouse *Qa* Region

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Introduction

The classical major histocompatibility complex (MHC) class I antigens are membrane glycoproteins with a molecular weight between 40,000 and 50,000 daltons which are associated with the secretory protein, β_2-microglobulin. These molecules have previously been defined using allo-antisera produced by cross-immunising mice differing at class I gene loci. Using such reagents, and later monoclonal antibodies, 7 class I molecules were described, H-2K,D,L,R, Qa-1, Qa-2 and TL. The advent of genomic DNA libraries and the mapping of large stretches of DNA within the MHC of man and mice showed that, in any one individual, the number of class I sequences far exceeds the number of serologically detectable class I molecules. In mice, most of the class I sequences map to the *Qa/Tla* region, suggesting that many of them may be 'pseudogenes' which do not encode expressable polypeptides. Later it became clear that some of these genes were transcribed and gave rise to class I polypeptides. This article summarises current knowledge on the structure and expression of molecules encoded in the mouse *Qa* region. Features shared between different Qa molecules suggest that they may comprise a family of proteins of biological importance.

Genetics

The discovery of the Qa-2 antigen [Flaherty, 1976] defined a new genetic locus between *H-2D* and *Tla*. Two recombinant mouse strains, B6.K1 and B6.K2, which are identical at the *Tla* and *H-2* loci, were found to differ at a new MHC locus. An allo-antiserum prepared by immunising B6.K1 mice with C57BL/6 thymus and lymph node cells defined an antigen, Qa-2, which was present on B6.K2 but not on B6.K1 cells. Further analysis of this antiserum showed that all inbred mouse strains typed as either *Qa-2*$^+$ or *Qa-2*$^-$. The *Qa-2* locus is now known to contain up to 10 class I genes [Weiss et al., 1984], designated *Q1* to *Q10* in C57BL/10

Table I. Properties of Qa antigens

Antigen	Reagent	Comments	References
Qa-2	B6.K1 anti-B6 (AS)	'classical' Qa-2, also contains anti Qa-3	Flaherty [1976]
	D3.262 (mc, IgM)	'classical' Qa-2	Lynes et al. [1982]
	Y-7 (mc, IgM)	'classical' Qa-2	Rucker et al. [1983]
	m2 (mc, IgG_2b)	'classical' Qa-2	Hogarth et al. [1982]
Qa-3	B6.K1 anti-B6 (AS) absorbed w. EL4 tumour	*Qa-2*$^+$ strains are all *Qa-3*$^+$ except SWR and DBA/1 *(H-2*^{q}*)*	Flaherty et al. [1978b]
Qat-4	(mc, IgM)	'classical Qa-2'	Hämmerling et al. [1979]
Qat-5	(mc, IgM)	only strains with *D/Qa* region from *H-2*^{b} are *Qa-5*$^+$	Hämmerling et al. [1979]
Qa-6	BALB/cBy anti-ORA-1 tumour (AS)	maps to *Tla* region, but is only expressed if Qa-2 is present	Widacki et al. [1985]
Qa-m7	(mc, IgM)	*Qa-2*$^+$ strains with *D/Qa* region from *H-2*^{a} are *Qa-7*$^-$	Sandrin et al. [1983]
Qa-m8	(mc, IgG_3)	*Qa-2*$^+$ strains with *D/Qa* from *H-2*^{d} or *H-2*^{a} are *Qa-8*$^-$	Sandrin et al. [1983]
Qa-m9	(mc, IgG_1)	like Qa-2, but requires β_2m^b for expression	Sutton et al. [1983]
Qa-11	(A.SW×C3H) F_1 anti-B10.A (2R) or (4R) (AS)	like Qa-6, but requires β_2m^b for expression	van der Meugheuvel et al. [1985]

AS = Antiserum; mc, IgM = monoclonal IgM; β_2m^b = 'b' allele of β_2-microglobulin. Qa-3-11 are only expressed in the presence of the Qa-2 antigen. The section 'Comments' indicates how the antigen differs from Qa-2.

mice. For consistency, the allelic equivalents in other mouse strains will be numbered according to the C57BL/10 nomenclature.

Several monoclonal antibodies against cell surface Qa antigens have been described. All of them react with B6.K2 but not with B6.K1 cells. Some differ, however, in their pattern of reactivity with other mouse strains. In addition, some anti H-2 allo-antibodies cross-react with *Qa* region molecules (Sharrow et al., 1984], reflecting a structural homology between H-2 and Qa antigens. The relationships between the antigenic determinants Qa-3 to Qa-11 have not yet been precisely defined, but it is notable that they are never expressed in the absence of Qa-2. It is therefore conceivable that Qa-3 to Qa-11 are all epitopes on molecules which also carry Qa-2 determinants. The properties of these reagents are summarised in table I. It is important to note that the designation of antigenic determinants is essentially chrono-

logical and is independent of the gene nomenclature. The genetics of the Qa-2 family of antigens has been reviewed by others [Flaherty, 1981; Flaherty et al., 1985; Harris et al., 1984].

Biochemistry

In addition to Qa-2, two other *Qa* region class I molecules, Q10 and Qb-1, have been studied in detail. The properties of all 3 molecules will be dealt with in turn.

The Qa-2 Molecule

Treatment of lysates of ^{125}I surface-labelled C57BL/6 spleen cells with the alloantiserum B6.K1 anti-B6 (anti-Qa-2) precipitates a heterodimeric molecule with a molecular weight of 40,000 and 12,000 daltons [Michaelson et al., 1981]. The 12,000-dalton component is identical in size and charge to mouse β_2-microglobulin, but the heavy chain of Qa-2 is about 5,000 daltons smaller than the heavy chains of H-2K^b and H-2D^b isolated from the same lysate. Peptide mapping and partial amino acid sequence analysis show a strong homology between Qa-2 and other class I molecules (Soloski et al., 1982]. No polymorphism was detected between Qa-2 polypeptides from several inbred mouse strains using SDS-PAGE and iso-electric focussing [Michaelson et al., 1982]. Furthermore, Qa-2 antigens isolated from *Mus musculus* and *Mus molossinus* subspecies were indistinguishable from those of laboratory strains. Although these experiments do not rule out genetic polymorphism, they do suggest that Qa-2 has a more conserved structure than H-2. We have found that the number of Qa-2 precursor forms detectable by 2-dimensional gel electrophoresis differs between strains [Robinson et al., in prep.]. The number of polypeptides observed appears to correlate with the number of genes encoding Qa-2 molecules. However, when processed to mature cell surface molecules, these strain-related differences are no longer evident. Heterogeneity of Qa-2 molecules from a series of BALB.B T lymphocyte clones [Sherman et al., 1984] was found not to be due to variation in N-linked carbohydrates but could be the result of differences in gene expression or mRNA splicing.

In addition to cell surface molecules, secreted forms of Qa-2 have been found. In one report, it was shown that activated, but not resting, C57BL/6 T lymphocytes secrete a molecule with Qa-2 antigenic determinants [Soloski et al., 1986]. The secreted molecule was similar in apparent molecular weight to cell surface Qa-2, but was more acidic. We have also detected a soluble Qa-2 molecule in supernates of activated T and B lymphocytes [Robinson, in press, 1987] which is 1,000–2,000 daltons smaller than the membrane form and is found labelled in supernates of ^{125}I surface-labelled lymphocytes. Pulse-chase experiments have shown that biosynthetically labelled Qa-2 molecules are detectable in culture supernates 45 min after synthesis and are released in a linear fashion for at least 3 h. These results are consistent with the hypothesis that newly synthesized Qa-2 molecules are expressed on the cell surface before being secreted. Thus, there are two views as to the mechanism of secretion. We propose that cell surface and secreted Qa-2 molecules are derived from a single precursor polypeptide. Soloski et al. [1986] favour the hypothesis that the membrane and soluble forms of Qa-2 are encoded by different mRNA transcripts, possibly originating from a single gene.

Partial digestion with endoglycosidase F shows that both the membrane and soluble Qa-2 molecules are glycoproteins with 2 asparagine-linked glycans [Robinson, in press, 1987]. In mouse class I molecules, glycosylation sites are found at asparagine residues 86,176 and 256. All H-2 molecules examined so far use the glycosylation sites at Asn86 and Asn176, and some molecules such as H-2K^d, H-2D^b and H-2D^k also use the Asn256 site [Maloy and Coligan, 1982]. In the nucleotide sequences of 3 class I genes encoding Qa-2-like polypeptides [Devlin et al., 1985b], the AAC sequence encoding Asn176 is altered to AAG for Lys, thereby eliminating the glycosylation site. Thus, Qa-2 is probably glycosylated at Asn86 and Asn 256.

Expression of Qa-2 antigens appears to be restricted to cells of the haematopoietic system [Flaherty et al., 1978a]. No Qa-2 was found in liver, brain or kidney. Proliferation of mouse spleen cells in response to the T cell mitogen concanavalin A was abrogated by treatment with anti-Qa-2 and complement, but the B cell response to lipopolysaccharide was not [Flaherty et al., 1978b], suggesting that Qa-2 is present on all T cells but not on all B cells. The level of expression of Qa-2 is also affected by genes in the H-2D region [Hogarth et al., 1983].

Another class I molecule, Qa-6, which maps to the *Tla* region, was indistinguishable from Qa-2 by 2-dimensional electrophoresis and peptide mapping [Widacki et al., 1985]. Although *Tla* may also encode Qa-2-like molecules, it is more likely that Qa-2 and Qa-6 are encoded by the same structural gene, but that expression of Qa-6 is controlled by a regulatory gene in the *Tla* region. Monoclonal antibodies defining the Qa-m2 and -m9 antigens also precipitate class I molecules with a molecular weight of 40,000 daltons [Hogarth et al., 1982; Sutton et al., 1983], but it has not yet been shown whether they are identical to the molecules detected using anti Qa-2 antisera.

The Q10 Molecule

Analysis of a mouse liver cDNA library revealed sequences encoding an unusual class I molecule [Kress et al., 1983]. Comparison of its nucleotide sequence with those of H-2 genes showed that 13 bases had been lost from exon 5, which normally encodes the membrane spanning segment of the protein. This gene would be expected to encode a protein in which several hydrophobic amino acids close to the C terminus had been replaced by charged or hydrophilic ones. The same exon 5 sequence was later found in the *Q10* gene from the *Qa* region of C57BL/10 mice [Mellor et al., 1984].

A rabbit antiserum raised against a synthetic peptide unique to Q10 and corresponding to amino acids 285–296 precipitates a soluble class I molecule with a molecular weight of 40,000 daltons which is associated with β_2-microglobulin [Maloy et al., 1984]. This molecule was found in serum and in liver, but not in spleen, thymus, kidney or testis. The amount of Q10 present in serum is strain-dependent and ranges from undetectable levels in B10.M(H–2^f) mice to 60 µg/ml in NZW [Lew et al., 1986]. The serum levels of Q10 are also influenced by the sex of the donors, males having more than females. Serum levels of Q10 also increase with age and decrease during pregnancy or after injection of irritants or syngeneic tumour cells. In serum, Q10 is part of a high molecular weight complex with a molecular weight of 200,000–300,000 daltons, and in this respect resembles an H-2-like

serum component described previously [Kvist and Peterson, 1979]. However, sequential immunoprecipitation studies now suggest that they are probably different [Maloy et al., 1984]. Q10 molecules display little or no genetic polymorphism and were found not to react with anti H-2^b allo-antisera [Maloy et al., 1984], although the N and C_1 domains of Q10 and H-$2K^b$ are about 85% homologous.

Digestion with endoglycosidase F shows that Q10 has 2 asparagine-linked glycans [Devlin et al., 1985a], and the nucleotide sequence predicts that they are attached at residues Asn86 and Asn256.

The Qb-1 Molecule

Recently, a new mouse class I polypeptide was identified in lysates of ^{35}S-labelled spleen cells by immunoprecipitation with antibodies against β_2-microglobulin and 2-dimensional gel electrophoresis [Robinson, 1985]. This molecule, Qb-1, is a glycoprotein with a molecular weight of 41,000 daltons and is associated with β_2-microglobulin. It occurs in at least 2 allelic forms, Qb-1^a and Qb-1^b, which are identical in size but differ in their iso-electric points. Most laboratory mouse strains are either *Qb-1*^{a} or *Qb-1*^{b}, but some strains did not synthesize Qb-1 and were designated *Qb-1*^{c}. By comparing 2-dimensional gel fingerprints from several recombinant haplotypes, it was possible to map the *Qb-1* gene to the *Qa* region.

Synthesis of Qb-1 was detected in several lymphoid tissues, including spleen, bone marrow, lymph nodes and in both B and T lymphocytes. Qb-1 does not react with most anti-H-2 allo-antisera or monoclonal allo-antibodies, but does react with rat antibodies directed against framework determinants of mouse class I molecules. One such antibody, R1.9.6., precipitates Qb-1 specifically from lysates of C57BL/6 spleen cells [Robinson et al., in prep.]. Using this antibody it could be shown that Qb-1 is secreted into the supernate and was not expressed on the cell surface. Partial endoglycosidase F treatment shows that Qb-1 has 2 N-linked glycans, as do Qa-2 and Q10. Transfection experiments have now established that the *Q4* gene encodes a polypeptide indistinguishable from Qb-1 (see later section).

Qa Molecules in Other Species

There have been reports of Qa-like molecules in man [Gazit et al., 1984], guinea-pigs [Schwartz et al., 1978] and rats [Haustein et al., 1982]. In most cases their similarity to Qa molecules has been based solely upon a restricted tissue distribution. However, for lack of a more general definition, it is difficult to say whether they are true homologues of mouse Qa antigens.

Common Features of Qa Molecules

Several structural characteristics distinguish Qa-2, Q10 and Qb-1 from other class I molecules. Firstly, the glycoprotein chains of all 3 molecules are 3,000–5,000 daltons smaller than those of H-2 molecules. This is almost certainly due to a shorter polypeptide chain, since that size difference is maintained after removal of N-linked glycans from both H-2 and Qa molecules using endoglycosidase F [Robinson, unpubl. data]. Since several *Qa* region genes have chain-termination sequences in or around exon 5, it is likely that their protein products would lack cytoplasmic domains. This would explain why Qa molecules appear to be smaller than H-2. Absence of a cytoplasmic domain may be a general feature of Qa molecules

and could be useful as a marker for homologous molecules in man and other species.

Qa-2, Q10 and Qb-1 all carry 2 asparagine-linked carbohydrate groups which in Q10 and probably also in Qa-2 are linked to residues Asn86 and Asn256. All published *Qa* sequences predict lysine at amino acid position 176, in place of asparagine found in all H-2 molecules.

Unlike other class I molecules, Qa-2, Q10 and Qb-1 can all be found in soluble form, with Qa-2 also occurring as a membrane-associated molecule. It is interesting to speculate that perhaps cell surface Qa-2 molecules may not themselves be functional, but may simply be precursors of the secreted forms.

Finally, a feature of all Qa molecules so far described is their restricted tissue distribution. Qa-2 is expressed only in haematopoietic cells, and Q10 is expressed only in liver. The possible functional relevance of this selectivity will be discussed later.

Expression of Qa Polypeptides and H-2/Qa Hybrid Molecules by Gene Transfer. The availability of cloned *Qa* region genes has enabled the identification of several polypeptides by gene transfer. A Qa-2-like molecule was expressed in mouse L cell fibroblasts after transfection of cosmid DNA from a BALB/c genomic library [Goodenow et al., 1982]. The exact identity of the molecule expressed is unclear, however, since the cosmid clone used, 50.2, has subsequently been mapped to the *H-2D* region [Stephan et al., 1986]. Most investigators, however, have been unable to detect cell surface expression of Qa molecules by direct transfection of *Qa* genes into fibroblasts.

To circumvent this problem, Stroynowski et al. [1985] constructed hybrid class I genes in which exons 1–3, encoding the N and C_1 domains of the protein, were derived from *Q6* or *Q7*, while exons 4–8, encoding the rest of the molecule, were from *H-2L*^{d}. High levels of hybrid products were found on the surface of L cells transfected with these constructs. If, however, exons 1–3 were derived from *H-2L*^{d} and exons 4–8 from *Q6* or *Q7*, no cell surface products were found. This experiment shows that sequences in the 3′ regions of *Q6* and *Q7* determine whether or not the corresponding products are expressed on the surface of L cells. There are two explanations for these results. Either the 3′ regions of *Q6/Q7* contain regulatory sequences which affect the rate of transcription in fibroblasts, or class I molecules with C termini from *Q6/Q7* are secreted.

We have transfected unmodified *Qa* genes into several different fibroblast lines and have used a short pulse of ^{35}S-methionine to label newly synthesized proteins. Products of transfected genes were detected by treatment of cell lysates with allo-antisera or with rat antibodies against framework determinants of mouse class I molecules. When immunoprecipitates were compared by 2-dimensional gel electrophoresis, no new molecules were detected in fibroblasts transfected with C57BL/10 cosmid DNA carrying the *Q1, Q2, Q3* or *Q5* genes. However, fibroblasts transfected with *Q4, Q6, Q7, Q8* and *Q9* were all found to synthesize polypeptides as a result of gene transfer. Moderate levels of Q4 product were synthesized by BALB/c3T3 cells transfected with *Q4* [Robinson et al., in prep.] and could be precipitated with R1.9.6 antibody. The Q4 molecule was found to comigrate in 2-dimensional gels with Qb-1 synthesized by C57BL/6 spleen cells, strongly suggesting that they are the same. Transfected fibroblasts were also found to secrete Q4 molecules into the culture medium.

Transfection of cloned *Q6, Q7, Q8* and *Q9* genes into L cells all gave rise to class I molecules reactive with anti-Qa-2 antisera [Mellor et al., 1985]. Only trace amounts of these polypeptides were found, however, and no Qa-2 antigens could be detected on the surface of transfected cells by immunofluorescence. The products of *Q6* and *Q8* have a molecular weight of 39,000–40,000 daltons and the *Q7* and *Q9* products of 35,000–36,000 daltons. According to their predicted amino acid sequences, the polypeptides encoded by *Q7, Q8* and *Q9* should differ in size by no more than 1,000 daltons [Devlin et al., 1985b]. These apparent anomalies could be explained by aberrant splicing of some mRNA transcripts or post-translational modification of the polypeptides. The single nucleotide difference between the *Q7* and *Q9* genes, CAG (glutamine) in *Q7*, GAG (glutamic acid) in *Q9* [Devlin et al., 1985b], is reflected in the iso-electric points of the corresponding polypeptides on 2-dimensional gels [Mellor et al., 1985]. It is not yet certain which of the *Q6–Q9* gene family encode the membrane and secreted forms of Qa-2 in C57BL/6 lymphocytes. However, since only *Q7* and *Q9* encode polypeptides with intact membrane-spanning domains, it is likely that one or both of these genes encode cell surface Qa-2 molecules. Comparison of the predicted amino acid sequences of the Q7–Q9 polypeptides with those of H-2 show multiple differences in the 2 outermost (N, C_1) protein domains. In the N domain, for instance, there are 15 amino acid differences between H-2K^b and Q7, and in C_1 there are 24 differences. Within the group, however, there are fewer differences, suggesting that Q7–Q9 molecules may carry multiple epitopes, some of which are shared by 2 or more gene products.

No Q10 mRNA transcripts could be detected in L cells transfected with unmodified cloned *Q10* genes. When transfected with the *Q10* gene under the control of the inducible mouse metallothionein I promotor, L cells synthesized and secreted Q10 molecules [Devlin et al., 1985a]. Thus, it appears that the difficulties in detecting Qa polypeptides after transfection of fibroblasts with *Qa* genes are due to regulatory elements associated with the transferred DNA.

Function

The function of Qa molecules is unknown. Several lines of evidence suggest, however, that their biological role, if any, is likely to be distinct from that of H-2. Firstly, whilst the number of *Qa* region genes varies between haplotypes, allelic genes and their products appear to be highly conserved. The degree of polymorphism of Qa molecules may thus be strictly controlled by selective pressures. Secondly, there is the question of tissue specificity. Expression of Q10 exclusively in liver and of Qa-2 only in haematopoietic cells is unlikely to be fortuitous. The liver produces several serum proteins, and the fact that Q10 molecules are produced only here suggests that they may be required in the blood. Since Qa determinants are expressed at different levels on haematopoietic progenitor cells they may be important for the development of certain lineages [Harris et al., 1984]. Attempts to demonstrate this have so far been unsuccessful. Modulation studies, performed by injecting Qa antibodies into newborn mice, succeeded in abolishing expression of the appropriate antigens, but failed to show any abnormalities in the development of the various lineages

Table II. C-terminal amino acid sequences of Q7, Q8, Q10 and H-2K^b, predicted from their nucleotide sequences [Devlin et al., 1985b; Mellor et al., 1984; Weiss et al., 1983]

	Q7	Q8	Q10	Q10*	K^b		Q7	Q8	Q10	Q10*	K^b
278	Glu	–	–	–	–	298	Ala	–	Leu	Leu	–
279	Pro	–	–	–	–	299	Val	Trp	Lys	Trp	Ala
280	Pro	–	–	–	–	300	Ala	Pro	Leu	Pro	Ile
281	Pro	–	–	–	–	301	Ile	Ser	Trp	Ser	Val
282	Tyr	Ser	Ser	Ser	Ser	302	Ile	Leu	Trp	Leu	Thr
283	Thr	–	–	–	–	303	Gly	Glu	Tyr	Lys	–
284	Val	–	Asp	Asp	–	304	Ala	Leu	Leu	Leu	–
285	Ser	–	–	–	–	305	Val	Trp	END	Trp	–
286	Asn	–	Ile	Ile	–	306	Val	Trp		Trp	–
287	Met	–	–	–	–	307	Ala	Ile		Tyr	–
288	Ala	–	Ser	Ser	–	308	Phe	Leu		Leu	–
289	Thr	Asn	His	His	–	309	Val	END		END	–
290	Ile	Val	–	–	Val	310	Met				–
291	Ala	–	–	–	–	311	Asn				Lys
292	Val	Ile	Asp	Asp	–	312	Arg				Met
293	Val	Leu	Leu	Leu	Leu	313	Arg				–
294	Val	–	Leu	*	–		END				Arg
295	Asp	Val	Trp	*	Val						Arg
296	Leu	–	Pro	*	–						Asn
297	Gly	Val	Ser	*	–						Thr

Numbers in the left-hand column denote Q7 amino acid positions. For Q8, Q10 and H-2K^b, the first amino acid is 275 rather than 278. 'Q10*' shows the Q10 sequence with spaces (*) added to maximise homology with Q7. Hyphens indicate that these amino acids are the same as in Q7. 'END' indicates chain termination.

[Flaherty et al., 1985]. Thus, the importance of Qa antigens as markers of haematopoietic differentiation has yet to be shown.

Thirdly, the fact that all Qa molecules so far described occur in soluble form raises the possibility that they may have a regulatory function within the immune system. Secreted Qa-2 and Qb-1, released by activated lymphocytes, may accumulate locally in lymphoid organs or at sites of inflammation. Since class I molecules are known to be involved in T cell recognition, soluble Qa molecules may modify T cell functions by interacting with antigen receptors and perhaps with the antigen itself. In the blood, soluble class I molecules (e.g. Q10) may associate with circulating antigen and present it to T cells in a recognisable form.

The ultimate destination of class I molecules is partly dependent upon their physical properties. The amino acid sequence around the membrane attachment site determines whether or not the polypeptide chain will be properly anchored in the membrane. In table II, the partial C-terminal amino acid sequences of Q8, Q10 and H-2K^b are compared with that of Q7. The membrane-spanning segment of the putative Q7 molecule is

similar to that of H-2K^b, the only important differences being a charged amino acid in position 295 of Q7 and its termination at position 313. It is therefore quite likely that Q7 is membrane-associated. The C-terminal sequence of Q8, however, is less hydrophobic than that of Q7, and is 5 amino acids shorter, suggesting that Q8 is secreted. The Q10 sequence is similar to Q8, but 4 more amino acids have been deleted, making the C terminus shorter still. Thus, at least three separate modifications have occurred in *Qa* region class I genes of C57BL/10 mice. All have occurred in exon 5 within a stretch of only 60 nucleotides, and each has a profound effect on the physical properties of the respective gene products. In this case, the result has been to make secreted proteins using genes which may have originally encoded cell surface molecules.

Conclusions

It is now clear that the *Qa* region in mice encodes several class I molecules. In C57BL/10 mice, at least 6 of the 10 class I genes at the *Qa* locus produce polypeptides. Some of the products are membrane-associated and some are secreted. As more Qa molecules are found, common structural features emerge. Sequence data show that they all have altered or truncated C termini, clearly distinguishing them from H-2 and other class I molecules. Their restricted tissue distribution suggests that they may be zonally distributed in the organism. It is now important to determine the molecular basis of this tissue specificity. With regard to their function, there are few clues at present, and we can only make correlations between the occurrence of Qa molecules and the cell types which produce them. The availability of large amounts of these molecules, produced by genetic manipulation, will enable us to test their properties in biological systems.

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