Coronavirus motif

SIR — I report the presence of a leucine zipper motif at the carboxyl end of the spike (S) glycoprotein, a transmembrane protein of coronaviruses. All the coronavirus S proteins whose sequences are known — transmissible gastroenteritis virus (TGEV) FS772/70 (residues 1,342–1,377), feline infectious peritonitis virus (FIPV) 79–1146 (1345–1380), mouse hepatitis virus (MHV) A59 (1217–1252), MHV JHM (1128–1163), human coronavirus (HCV) 229E (1,067–1,102), bovine coronavirus (BCV) Mebus (1,266–1,294) and infectious bronchitis virus (IBV) Beaudette (1,058–1,079) — contain a

leucine-zipper motif terminating 10 amino-acid residues upstream of the conserved KWP motif preceding the transmembrane domain.

The length of the leucine zippers range from three heptad repeats, as identified for the F glycoprotein of paramyxoviruses, to five heptad repeats. The observation that all coronavirus S proteins sequenced so far contain the leucine-zipper motif 10 amino-acid residues from the transmembrane domain may imply some function of the motif in the dimerization of the S polypetides.

PAUL BRITTON

Institute for Animal Health,
Compton Laboratory,
Compton, Newbury RG16 0NN, UK

Virus	N° of amino acid residues	Leucine Zipper motif		Transmembrane Domain
		* * * * * *		
TGEV FS772/70	1149	1341 - NLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVV	-	1401
PRCV 86/137004	1225	1117 - NLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVV	-	1177
FIPV 79-1146	1452	1344 - NLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNLEWLNRIETYVKWPWYVWLLIGLVV	-	1404
MHV A59	1325	1216 - DLSLDFEKLNVTLLDLTYEMNRIQDAIKKLNESYINLKEVGTTEMYVKWPWYVWLLIGLAG	-	1276
MHV JHM	1235	1127 - DLSLDFEKLNVTFLDLTYEMNRIQDAIKKLNESYINLKEVGTYEMYVKWPWYVWLLIGLAG	-	1187
HCV 229E	1173	1066 - NLTSEISTLENKSAELNYTVQKLQTLIDNINSTLVDLKWLNRVETYIKWPWWVWLCISVVL	-	1126
BCV Mebus	1363	1265 - YINVTFLDLQDEMNRLQEAIKLLNQSYINLKDIGTYEYYVKWPWYVWLLIGFAG	-	1318
IBV Beaudette	1162	1057 - DIDSEIDRIQGVIQGLNDSLIDLEKLSILKTYIKWPWYVWLAIAFAT	-	1103
		* * * * * *		

Comparison of the leucine-zipper motifs found in the different coronavirus S proteins. The KWP motif preceding the potential transmembrane domain and the transmembrane domains are also shown. The repeated leucine or isoleucine residues are marked with an asterisk. PRCV, porcine respiratory coronavirus.

Biocontrol risks

SIR — Hochberg and Waage suggested in their News and Views article[1] that some new genetically modified insect viruses will be acceptable as biological control agents because they have "highly restricted host ranges". There is widespread agreement that specific biological control agents are much to be preferred, on environmental grounds, over chemical pesticides. But the dangers of non-specific biocontrols are great, and much damage has resulted from their use[2].

How specific are these genetically modified organisms? They are derived from the *Autographa californica* nuclear polyhedrosis virus (AcNPV), a baculovirus, which has a wide and sporadic host range in the lepidoptera. There are around 2,500 species of lepidoptera in Britain[3], and of course many times more elsewhere. The records of host range of this virus[4–6], based on a small fraction of the known species, show that, of twelve superfamilies tested. eight apparently contain 'permissive' species (species killed by fewer virus polyhedra than are produced by one dead caterpillar). With this and other baculoviruses[7] one species in a genus may be permissive, others resistant and the LD50s vary markedly between different permissive species, without apparent regard for taxonomy.

The superfamilies known to have permissive species are the Gelichiodea, Pyraloidea, Papilionoidea, Sphingoidea and Noctuoidea: the Bombycoidea, Geometroidea and Yponometroidea may have them, but this needs confirmation by DNA analysis. It seems that 5 – 10 per cent of British lepidoptera are permissive for AcNPV, a non-native virus, putting 125–250 species at risk, including some of great conservation value.

The two new genetically modified organisms[8,9], like others derived from the same virus[10], may have host ranges slightly different from that of the wild type. But unless they can be further engineered to be absolutely specific for a known set of (pest) species, it is difficult to see that they could be used safely in an uncontrolled way in the field. Would not the risk assessment required under EC Directive 90/220, for instance, indicate that they are undesirable?

Hochberg and Waage[1] also note that "disabling engineered viruses so that they do not persist has some appeal". Removing the polyhedrin gene to produce a non-occluded virus[5,10] reduces both persistence and, to a small extent, the host range; neither of the two new genetically modified organisms has apparently been modified in this way.

Research into the molecular and other

bases of host specificity is likely to be a *sine qua non* for the successful, much-desired, replacement of chemical control agents for insects by viral ones.

MARK WILLIAMSON

Department of Biology,
University of York,
York YO1 5DD, UK

1. Hochberg, M. E. & Waage J. K. *Nature* **352**, 16—17 (1991).
2. Howarth, F. G. *A. rev. Ent.* **36**, 485 – 509 (1991).
3. Bradley, J. D. & Fletcher, D. S. *An indexed List of British Butterflies and Moths* (Kedleston, Orpington, 1986).
4. Payne, C. C. *Fortschr. Zool.* **32**, 183 – 200 (1986).
5. Bishop, D. H. L., Entwistle, P. F., Cameron, I. R., Allen, C. J. & Possee, R. D. in *The Release of Genetically-engineered Micro-organisms* (eds Sussman, M., Collins, C. H., Skinner, F. A. & Stewart-Tull, D. E.) 143–179 (Academic, London, 1988).
6. Réchaussat, L. & Williamson, M. H. *Final Report on a Feasibility Study on the Establishment of European Biotechnology Environmental Release Database* (BAP, CEC DGXII, Brussels, 1989).
7. Allaway, G. P. & Payne, C. C. *Ann. appl. Biol.* **105** 29–37 (1984).
8. Tomalski, M. D. & Miller, L. K. *Nature* **352**, 82–85 (1991).
9. Stewart, L. M. D. *et al. Nature* **352**, 85–88 (1991).
10. Bishop, D. H. L. & Possee, R. D. *C. R. Acad. Agric. Fr.* **75**, 157–168 (1989).

Ionicity in silica

SIR — Kramer *et al.*[1] suggest that a change in ionicity is responsible for the transition from the α to the β phase in silica. Their *ab initio* force-field method indicates that an increase by about 0.1 atomic units of the net charge on silicon stabilizes the β structure of quartz and cristobalite with respect to the α structures, implying that the former correspond to global minima of the Born–Oppenheimer potential-energy surface. Our recent Hartree–Fock calculations[2] on the quartz structures of SiO_2 and GeO_2 come to opposite conclusions with respect to both the magnitude and the direction of the ionicity effect.

Our calculations were performed at a level of accuracy similar to that of the cluster calculations used to derive the force field of ref. 1. We used the $P3_22_1$ space group for both the α and the β structures. In α quartz there are two equivalent twinned configurations, α_1 and α_2, related by a rotation of the SiO_4 tetrahedra around their C_2 axes. The corresponding internal coordinate, the tilt angle δ (ref. 3), is negative in the α_1 phase, positive in α_2 and zero in β.

The net charges calculated by this method should provide reliable trends of ionicity in connected structures when identical basis sets are used. We find that the net charge on silicon decreases

1. Kramer, G. J., van Beest, B. W. H. & van Santen, R. A. *Nature* **351** 636–638 (1991).
2. Silvi, B., D'Arco, Ph. & Causà, M. *J. chem. Phys.* **93**, 7225–7229 (1990).
3. Grimm, H. & Dorner, B. *J. Phys. Chem. Solids* **36**, 407–413 (1975).
4. Tsuneyuki, S., Aoki, H., Tsukada, M. & Matsui, Y. *Phys. Rev. Lett.* **64**, 776–779 (1990).
5. Tezuka, Y., Shin, S. & Ishigame, M. *Phys. Rev. Lett.* **66**, 2356–2359 (1991).
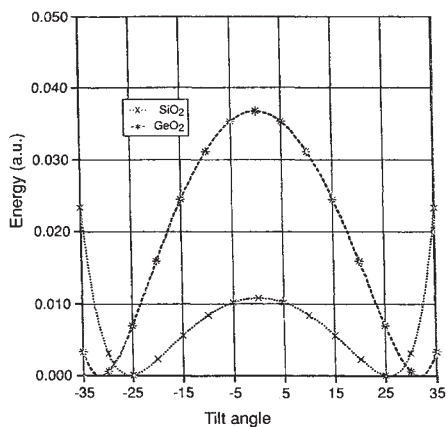
Unit-cell energy (a.u.) versus tilt angle for cristobalite SiO_2 (dotted line) and GeO_2 (dashed line). The origin of the potential corresponds to the α twinned structure.

from the α to the β structure by 0.018 a.u. in quartz and 0.014 a.u. in cristobalite. In fact, no net changes in ionicity between any of the crystal phases are predicted by our *ab initio* calculations on infinite crystals.

The Born–Oppenheimer energy curve $E(\delta)$ suggests that the high-temperature β phase can be viewed not as a minimum of the potential-energy surface but rather in terms of the expectation values of the atomic coordinates with respect to the nuclear wavefunction. Molecular dynamics calculations[4] provide support for this interpretation. Starting from the α_1 structure, the α_2 twin appears as the temperature is raised, and at 850 K the system alternates dynamically between the two phases. This corresponds to the appearance of a symmetric double-well potential in the Born–Oppenheimer energy surface (see figure). The soft-mode normal coordinate can be approximated by the tilt angle δ. For the symmetric double well, the expectation value $<\delta>=0$, corresponding to the β phase, whereas at low temperature the symmetry is broken and $<\delta>$ takes negative and positive values for α_1 and α_2, respectively. When the nuclei are considered as quantum particles, the $\alpha \rightarrow \beta$ transition is displacive, in agreement with experiments[5], whereas a classical description corresponds to the dynamical picture of the molecular dynamics calculation[4].

As pointed out by Tsuneyuki *et al.*[4], the thermal energy required to symmetrize the potential increases with the height of the barrier separating the twinned structures. We calculate a higher barrier for GeO_2 than for SiO_2 (see figure), which explains why no β structure is observed for the germanium analogues of silica polymorphs.

BERNARD SILVI

Laboratoire de Dynamique des
Interactions Moléculaires (UR271),
Université Pierre et Marie Curie,
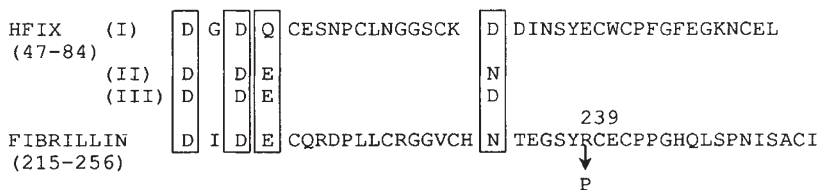*4 place Jussieu, 75230 Paris, France*
PHILIPPE D'ARCO
MAURO CAUSÀ
Laboratoire de Géology de l'Ecole
Normale Supérieure (URA1316),
24 rue Lhomond,
75231 Paris, France

where *notch* and *delta*, two *Drosophila* proteins containing EGF-like repeats with the calcium-binding consensus, interact in a calcium-dependent manner. Could calcium interaction with EGF-like repeats in fibrillin be intimately linked with its function in connective tissue?

P. A. HANDFORD
M. MAYHEW
G. G. BROWNLEE
Chemical Pathology Unit,
Sir William Dunn School of Pathology,
South Parks Road,
Oxford OX1 3RE, UK

1. Dietz, H. C. et al. Nature 352 337–339 (1991).
2. Maslen, C. L. et al. Nature 352, 334–337 (1991)
3. Handford, P. A. el al. Nature 351, 164–167 (1991)
4. Baron, M. et al. Trends biochem. Sci 16, 13–17 (1991)
5. Fehon, R. G. et al. Cell 61, 523–534 (1990)

Bell's inequality

SIR — Maddox's article[1] on non-locality in quantum mechanics reminded me that Bell's inequality has a long history which may be of some interest. A few years ago, I attempted to trace the origins of the probability metric. (The distance between two events is the probability that one of them occurs but not both. Strictly speaking, this is a pseudo-metric from which a metric can be constructed by standard techniques.) My colleague, D. A. Edwards, pointed out that the treatise by Dunford and Schwartz[2] contains both a discussion of a more general metric on measure spaces and a survey of its history. There it is traced back to work of Aronszajn and Nikodým[3] in the late 1920s. Moreover, that metric is actually just a special case of the L^1 metric on integrable functions discussed 10 years earlier by Fréchet[4]. (One restricts the L^1 metric to indicator functions of events.)

The fact that the basic inequality was known for so long makes it all the more surprising that, until Bell's independent rediscovery of it in the 1960s, no one seems to have observed that the triangle inequality fails in quantum mechanics and can therefore provide a test of large classes of hidden variable theories. The new paper by Fivel[5], which Maddox discusses, provides an interesting new insight by comparing the probability metric with the quantum-mechanical Hilbert space metric and thereby isolating the mechanism which gives rise to Bell's inequality in one case but not in the other.

KEITH C. HANNABUSS

Balliol College,
Oxford OX1 3BJ, UK

1. Maddox, J. Nature 352, 277 (1991).
2. Dunford, N. & Schwartz, J. T. Linear Operators I General Theory (Wiley Interscience, New York 1958).
3. Nikodým, O. M. C. R. Congrés des Mathematiciens des Pays Slaves 304–313 (Warsaw, 1929).
4. Fréchet, M. Bull. Calcutta Math. Soc. 11, 187–206 (1919).
5. Fivel, D. Phys. Rev. Lett. 67 286 (1991).

Calcium binding to fibrillin?

SIR — Dietz *et al.*[1] described an Arg 239→Pro mutation in one of the 34 epidermal growth factor (EGF)-like repeats of the filbrillin gene[2] found in two patients with Marfan syndrome. These repeats contain a consensus sequence (see diagram) analogous to that found in the first EGF-like domain of coagulation factor IX which is known to bind calcium[3]. It therefore seems likely that fibrillin will also bind calcium. Moreover, the two-dimensional NMR structure of the EGF-like domain[4] of factor IX suggests that Arg 239 in the analogous fibrillin EGF-like domain is located on the second strand of an antiparallel β-sheet adjacent to the inferred calcium binding site. We propose that local disruption of this calcium-binding site could be responsible for the defect in fibrillin function, although long-range effects of the proline substitution on protein structure cannot be excluded. Supporting the concept of local disruption is the identification of two mutations in the factor IX EGF-like domain, Asp 47→Glu and Asp 64→Asn, present in patients with haemophilia B. These mutations reduce calcium binding appreciably but do not grossly affect the protein's structure[3].

Several groups have proposed that EGF-like domains are involved in protein–protein interactions. We would like to draw attention to a recent paper[5]

```
HFIX     (I)   D  G  D  Q  CESNPCLNGGSCK  D  DINSYECWCPFGFEGKNCEL
(47-84)
         (II)  D     D  E                 N
         (III) D     D  E                 D
                                                239
FIBRILLIN      D  I  D  E  CQRDPLLCRGGVCH  N  TEGSYRCECPPGHQLSPNISACI
(215-256)                                      ↓
                                               P
```

Amino acid residues important for calcium binding to the human factor IX (HFIX) EGF-like domain (I) are shown boxed with the analogous residues from one EGF-like domain of fibrillin. The position of the mutation Arg 239→Pro identified in two patients with Marfan syndrome is indicated. Variations of the calcium-binding consensus found in other proteins, which also bind calcium when introduced into factor IX are shown (II, III).