Science
AAAS

Influenza Escapes Immunity Along Neutral Networks

Erik van Nimwegen

Science **314**, 1884 (2006);
DOI: 10.1126/science.1137300

hexapods and malacostracan crustaceans (crabs and crayfish) (*15*, *16*), recent molecular sequence data suggest that hexapods are closely related to branchiopods (*17, 19*, *20*), a freshwater dwelling group of crustaceans that includes water fleas and fairy shrimp. This hypothesis is supported by analysis of Hox genes that demonstrates homology between development of the pregenital trunk region in insects and the thorax in branchiopods (*21*). The new molecular results correspond well with the fossil record and suggest an evolutionary origin of the hexapods in freshwater around 410 million years ago rather than in the marine Cambrian environment (*17*).

The vast majority of extant branchiopods are freshwater animals, and the few that are found in saltwater are believed to have invaded the sea secondarily. From the fossil record, it is known that modern branchiopods date back to the Early Devonian, by which time they were fully adapted to freshwater habitats (*22*). This late appearance of the freshwater branchiopods corresponds exactly with the emergence of hexapods and suggests that their last common ancestor swam around in a freshwater pond sometime in the Late Silurian (423 to 416 million years ago) or Early Devonian. This corresponds well with the time split between the crustacean and hexapod lineages estimated from molecular clock analyses (*23*). If correct, the early marine ancestor of the hexapods might have appeared more similar to *Rehbachiella kinnekullensis*, a close marine relative to branchiopods from Upper Cambrian (*24*), than to *D. bocksbergensis* or other hexapods.

The successful colonization of the terrestrial environment by hexapods seems to coincide with other major groups of land pioneering animals such as the chelicerates and the myriapods in the Late Silurian and the tetrapods (amphibians, reptiles, birds, and mammals) in the Late Devonian. All these events appear to have occurred through a freshwater dwelling phase in their evolutionary transition from marine to true terrestrial animals. The Devonian is believed to have been a time of severe drought, which might have forced these animals (at least hexapods and tetrapods) onto land as their freshwater habitats vanished.

It has been a puzzle as to why hexapods—in particular insects, which possess a morphology that apparently enables them to adapt to virtually all types of terrestrial environments—have not been able to diversify successfully in the marine environment. It is likewise remarkable that the crustaceans—fulfilling a biological role in the sea comparable to the insects on land—have not been able to invade land to a greater extent despite their considerable age. The recent phylogenetic analyses of molecular sequence data suggest a paradigm shift concerning the phylogenetic position of hexapods—that crustaceans successfully invaded land as insects. It is possible that when insects entered terrestrial habitats, their crustacean ancestors had already diversified in marine environments and occupied all potential niches, which could explain why insects were prevented from colonizing the sea subsequently. Most important, however, the new molecular results offer a solution to the enigma concerning the absence of marine hexapod remains in the fossil records prior to the Devonian.

References and Notes

1. G. Giribet, C. Ribera, *Cladistics* **16**, 204 (2000).
2. J. M. Mallatt *et al.*, *Mol. Phy. Evol*. **31**, 178 (2004).
3. J. C. Regier, J. W. Shultz, *Mol. Phy. Evol*. **20**, 136 (2001).
4. J. W. Shultz, J. C. Regier, *Proc. R. Soc. London Ser. B* **267**, 1011 (2000).
5. U. W. Hwang *et al.*, *Nature* **413**, 154 (2001).
6. G. Giribet *et al.*, *Nature* **413**, 157 (2001).
7. C. E Cook *et al., Curr. Biol.* **11**, 759 (2001).
8. Y.-x. Luan *et al.*, *Mol. Biol. Evol*. **22**, 1579 (2005).
9. W. Dohle, *Ann. Soc. Ent.* France **37**, 85 (2001)
10. H. Dove, A. Stollewerk, *Development* **130**, 2161 (2003).
11. G. E. Budd *et al.*, *Science* **294**, 2047a (2001).
12. F. Hass *et al.*, *Org. Divers. Evol.* **3**, 39 (2003).
13. R. Willmann, *Org. Divers. Evol*. **5**, 199 (2005).
14. M. S. Engel, D. A. Grimaldi, *Nature* **427**, 627 (2004).
15. S. Harzsch, *Integrative Comp. Biol.* **46**, 162 (2006).
16. N. J. Strausfeld, *Arthropod Struct. Dev.* **34**, 235 (2005).
17. J. C. Regier, J. W. Shultz, *Mol. Biol. Evol.* **14**, 902 (1997).
18. C. E. Cook, Q. Y. Yue, M. Akam, *Proc. R. Soc. London Ser. B* **272**, 1295 (2005).
19. J. C. Regier *et al.*, *Proc. R. Soc. London Ser. B* **272**, 395 (2005).
20. J. Mallatt, G. Giribet, *Mol. Phy. Evol.* **40**, 772 (2006).
21. M. Averof, M. Akam, *Nature* **376**, 420 (1996).
22. S. R. Fayers, N. H. Trewin, *Trans. R. Soc. Edinburgh Earth Sci.* **93**, 355 (2003).
23. M. W. Gaunt, M. A. Miles, *Mol. Biol. Evol*. **19**, 748 (2002).
24. D. Walossek, *Fossils Strata* **32**, 54 (1993).

25. This work was supported by The Danish Natural Science Council, The Velux Foundation, The Carlsberg Foundation, and The Wellcome Trust.

10.1126/science.1129844

EPIDEMIOLOGY

Influenza Escapes Immunity Along Neutral Networks

Erik van Nimwegen

Given that influenza virus continues to escape immunity, why is it that only one strain dominates each year? The answer may lie in neutral networks and mapping viral genotypes to antigenic phenotypes.

In the late 1960s, Kimura (*1*) made the then-revolutionary proposal that many amino acid substitutions are neutral in terms of evolutionary selection. There is now little doubt that essentially any genotype can undergo a substantial number of amino acid substitutions without substantially changing its fitness. This implies that there are large collections of selectively neutral genotypes that are connected through point mutations. Indeed, such "neutral networks" are observed in genotype-to-phenotype mappings of biomolecules (*2*, *3*). On page 1898 of this issue, Koelle *et al*. (*4*) provide compelling evidence that neutral networks play a key role in the evolution of human influenza A (H3N2).

Computer simulations and analytical studies (*5*–*7*) have shown that intertwined neutral networks have profound consequences for evolutionary adaptation. Evolving populations typically exhibit "epochs" of phenotypic stasis, punctuated by sudden changes in phenotype. However, phenotypic stasis is not accompanied by genotypic stasis. During each phenotypic epoch, the population is dominated by genotypes belonging to one neutral network, and neutral mutations cause the population to drift continuously through this neutral network (see the figure). Mutations to neighboring neutral networks (those networks that can be reached by a point mutation from one of the genotypes in the current neutral network) occur as well and enable the population to explore other phenotypes until, eventually, a mutant on a neutral network with higher fitness is generated. The offspring of this beneficial mutant will then spread through the population, causing a sudden shift in phenotype. Until the study by Koelle *et al*., this "epochal evolution" scenario (see the figure) had been observed mostly in silico and from in vitro evolution experiments (*5*–*8*).

The author is in the Division of Bioinformatics, Biozentrum, University of Basel, 4056 Basel, Switzerland. E-mail: erik.vannimwegen@unibas.ch

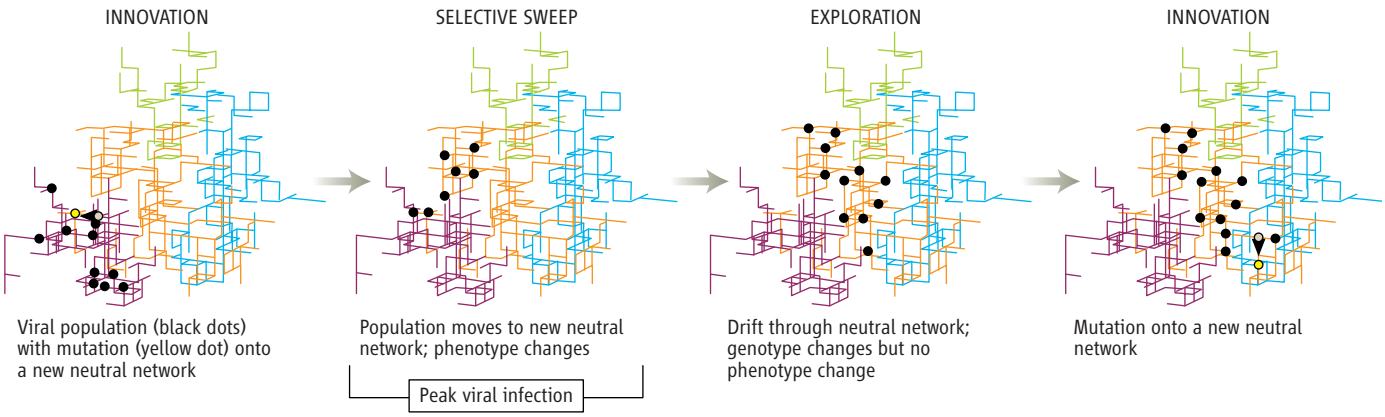
One of the main influenza antigens targeted by our immune system is the viral surface protein hemagglutinin (HA), which binds to host cells. The virus is therefore under strong evolutionary pressure to alter the shape of its HA protein (*9*) so as to escape the immune response. A major breakthrough in understanding this dynamic came from measuring cross-immunity between different strains of influenza (*10*). The study produced "antigenic maps" in which each viral strain corresponds to a point in an abstract "antigenic space" and the distance between points quantitatively reflects the amount of cross-immunity between the corresponding strains. Strains of influenza A (H3N2) separate into distinct clusters in antigenic space, each cluster corresponding to the set of strains from a particular time period. Whereas the genetic distance of strains to the ancestral strain increases smoothly, periods of relative phenotypic stasis (corresponding to antigenic clusters) are punctuated by sudden transitions from one antigenic cluster to another. Koelle *et al*. studied the genetic differences between strains from different antigenic clusters and provide evidence that antigenic clusters correspond to neutral networks. In particular, they find that there is no simple relationship between antigenic and genetic distance and no simple relation between changes at particular amino acids and cluster transitions.

To model influenza A (H3N2) evolution, Koelle *et al*. couple an epidemiological model that treats individuals as susceptible to, infected by, or recovered from each of the existing strains (*11*) with a neutral network model (*12*) for mapping genotypes to antigenic phenotypes. The figure illustrates the epochal evolution cycle exhibited by this model. At the start of the epochal evolution cycle, the viral population is distributed over the neutral network of one antigenic cluster (purple) and a mutation creates an individual on another neutral network (orange). Because a substantial fraction of the host population is no longer susceptible to strains from the purple neutral network at this time, the orange mutant has a high fitness advantage, and its descendants take over the viral population in a selective sweep. This "innovation" is associated with a peak in infections. After this phase, the viral population starts an exploratory phase in which it drifts over the orange neutral network, away from the ancestral strain, increasing its genotypic diversity without a substantial change in phenotype, while the fraction of hosts that are susceptible to strains from the orange neutral network declines. Eventually a mutant is created on a new neutral network (blue) and the cycle repeats itself.

The model of Koelle *et al.* elegantly explains several observed features of the evolution of influenza A (H3N2). First, the model produces phylogenetic trees that are structured much like those observed for influenza, a feature that has been one of the main puzzles regarding influenza evolution (*13*). Second, an innovation typically causes a strong peak in infections. Third, because every innovation involves one or very few mutants, genetic diversity in the population will drop steeply at each cluster transition, after which the genetic diversity will rebound during the exploration phase of the epochal cycle.

These results indicate that the structure and interconnectivity of the neutral networks corresponding to different antigenic clusters of HA are key features determining influenza A (H3N2) evolution. In silico (*14*) and in vitro evolution studies (*15*) have suggested that by combining data on protein structure with sequence data and computational modeling, some of the structural features of neutral networks can be reconstructed. For example, probably a major constraint shaping HA neutral networks is the requirement to maintain HA's affinity to the host receptor that it binds. In addition, much is understood about how parameters such as mutation rates and effective population sizes affect epochal evolutionary dynamics (*16*), and this should enable more detailed modeling of influenza A's evolutionary dynamics. For species with high mutation rates, such as RNA viruses, it has been established both theoretically (*17*) and from sequence analysis (*18*) that populations do not drift randomly through the neutral networks but rather tend to converge to the most connected areas of the neutral network. These areas typically also correspond to sequences that fold robustly into their target shape (*19*).

Epochal evolution may also occur in the evolution of other pathogens. Particularly interesting in this regard is the fact that the intrahost phylogenetic trees of HIV and hepatitis C virus (HCV) show topologies that are very similar to those observed for influenza A (H3N2) (*20*). The key to investigating the possible role of epochal evolution in these systems will be to develop a quantitative antigenic understanding—for example, by reconstructing antigenic maps for HIV and HCV strains, as was done for influenza strains.



Cycling through networks. Schematic representation of the epochal model of influenza A evolution. Entangled neutral networks of genotypes (sets of genotypes, each with the same antigenic phenotype) are connected by single point mutations. By drifting through and switching between these neutral networks, the viral population exhibits periods of phenotypic stasis punctuated by sudden phenotypic transitions.

References

1. M. Kimura, *Nature* **217**, 624 (1968).
2. K. F. Lau, K. A. Dill, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 638 (1990).

3. P. Schuster, Fontana, P. F. Stadler, I. L. Hofacker, *Proc. Biol. Sci.* **255**, 279 (1994).
4. K. Koelle, S. Cobey, B. Grenfell, M. Pascual, *Science* **314**, 1898 (2006).
5. M. A. Huynen, P. F. Stadler, W. Fontana, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 397 (1996).
6. E. van Nimwegen, J. P. Crutchfield, M. Mitchell, *Phys. Lett. A* **229**, 144 (1997).
7. W. Fontana, P. Schuster, *Science* **280**, 1451 (1998).
8. S. F. Elena, V. S. Cooper, R. E. Lenski, *Science* **272**, 1802 (1996).
9. R. M. Bush, C. A. Bender, K. Subbarao, N. J. Cox, W. M. Fitch, *Science* **286**, 1921 (1999).
10. D. J. Smith *et al.*, *Science* **305**, 371 (2004); published online 24 June 2004 (10.1126/science.1097211).
11. J. R. Gog, B. T. Grenfell, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 17209 (2002).
12. M. E. J. Newman, R. Engelhardt, *Proc. Biol. Sci.* **265**, 1333 (1998).
13. W. M. Fitch, R. M. Bush, C. A. Bender, N. J. Cox, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7712 (1997).
14. U. Bastolla, M. Porto, M. H. Eduardo Roman, M. H. Vendruscolo, *J. Mol. Biol.* **56**, 243 (2003).
15. C. A. Voigt, S. L. Mayo, F. H. Arnold, Z. G. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3778 (2002).
16. E. van Nimwegen, J. P. Crutchfield, M. Mitchell, *Theor. Comp. Sci.* **229**, 41 (1999).
17. E. van Nimwegen, J. P. Crutchfield, M. A. Huynen, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9716 (1999).
18. A. Wagner, P. F. Stadler, *J. Exp. Zool.* **285**, 119 (1999).
19. E. Bornberg-Bauer, H. S. Chan, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 10689 (1999).
20. B. T. Grenfell *et al.*, *Science* **303**, 327 (2004).

10.1126/science.1137300

BIOCHEMISTRY

If the RNA Fits, Use It

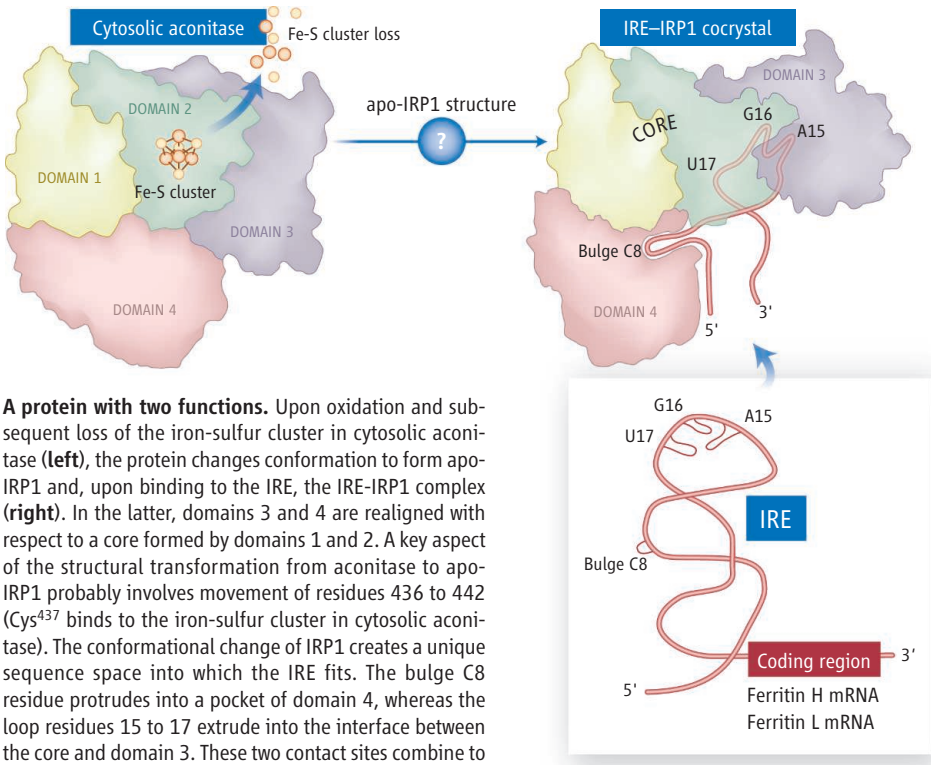
A crystal structure of RNA bound to the IRP1 protein explains how this protein can perform two entirely different functions.

Tracey A. Rouault

Cells and organisms use a wide variety of regulatory mechanisms to sense and respond to changes in the extracellular environment. Cells can regulate gene expression at several steps after a gene has been transcribed. On page 1903 of this issue, Walden *et al.* (*1*) shed light on one such posttranscriptional regulatory mechanism. They show how a single protein—iron regulatory protein 1 (IRP1)—responds to changing conditions by performing two entirely different functions.

In iron-replete cells, IRP1 functions as an aconitase enzyme, which interconverts citrate and isocitrate in the cytosol (*2*, *3*). However, in cells that are iron-depleted and oxidatively stressed, the fragile and exposed iron-sulfur cluster in the aconitase active site disassembles and is lost from the protein. The protein transforms into apo-IRP1, which can bind RNA stem-loops within transcripts of iron metabolism genes known as iron-responsive elements (IREs) (see the figure) (*4*). Walden *et al.* now report the crystal structure of IRP1 bound to a ferritin IRE. Together with the previous structure of cytosolic aconitase (*3*), this cocrystal structure reveals how apo-IRP1 binds with high affinity to IREs, whereas cytosolic aconitase does not.

Cytosolic aconitase has four domains. Residues from each domain contribute to the enzymatic active site, including three cysteines that bind to the iron-sulfur cluster. In the IRE-IRP1 cocrystal, domain 4 has moved and rotated relative to its position in cytosolic aconitase (see the figure). Domain 3 has also substantially shifted its position relative to the central core formed by domains 1 and 2, opening up a hydrophilic cavity between the core and domain 3.



A protein with two functions. Upon oxidation and subsequent loss of the iron-sulfur cluster in cytosolic aconitase (**left**), the protein changes conformation to form apo-IRP1 and, upon binding to the IRE, the IRE-IRP1 complex (**right**). In the latter, domains 3 and 4 are realigned with respect to a core formed by domains 1 and 2. A key aspect of the structural transformation from aconitase to apo-IRP1 probably involves movement of residues 436 to 442 (Cys^{437} binds to the iron-sulfur cluster in cytosolic aconitase). The conformational change of IRP1 creates a unique sequence space into which the IRE fits. The bulge C8 residue protrudes into a pocket of domain 4, whereas the loop residues 15 to 17 extrude into the interface between the core and domain 3. These two contact sites combine to establish specific high-affinity binding of the IRE to IRP1.

Previous structural and mutagenesis studies of IREs, which have conserved structural and sequence elements but are not identical in different transcripts, indicated that the most important residues for high-affinity binding to IRP1 would be the unpaired residues of the terminal loop and an unpaired cytosine that interrupts the double-helical structure of the upper and lower stems. In the cocrystal, these unpaired residues contribute to two discrete binding sites between the IRE and IRP1. In one site, the terminal-loop residues A15, G16, and U17 interact with residues in the cavity between the core and domain 3. In the second binding site, separated by 1.0 nm from the first, the C8 bulge residue fits into a pocket of domain 4 (see the figure).

The structure of the IRE in the complex is similar to its structure in solution, except that the purine bases of the terminal loop (residues A15 and G16) reorient from a tucked position to extrude into the cavity between the core and domain 3. IRE is thus an ideal binding partner for apo-IRP1, because it can bind to apo-IRP1 while minimally reorganizing its terminal loop.

Apo-IRP1 accumulates in cells that cannot

The author is with the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, Bethesda, MD 20892, USA. E-mail: trou@helix.nih.gov