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Animal genomics in natural reservoirs of infectious diseases

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Summary

Natural virus reservoirs such as wild bats, birds, rodents and non-human primates are generally non-model organisms that have, until recently, presented limited opportunities for in-depth study. Next-generation sequencing provides a way to partially circumvent this limitation, since the methods required for data acquisition and analysis are largely species-independent. Comparative genomics and other 'omics' provide new opportunities to study the structure and function of various biological systems of wild species that are otherwise out of reach. Genomes of natural reservoir hosts can help to identify dominant pathways of virus–host interaction and to reveal differences between susceptible and resistant organisms, populations and species. This is of great scientific interest and may also provide a resource for the rational design of treatments for viral diseases in humans and livestock. In this way, we will 'learn from nature' and one day apply this knowledge to create disease-resistant livestock or develop novel therapeutic and prevention strategies. Reservoir host genomics will also open up possibilities for developing novel vaccines for wildlife, aid in the development of new diagnostic platforms, and have broad implications for developmental and evolutionary biology. In this review, the authors focus on natural reservoir hosts of viral pathogens, although most of the discussion points should be equally applicable to natural reservoirs of pathogenic bacteria, fungi or other parasites.

Keywords

Bat – Bird – Genomics – Next-generation sequencing – Non-human primate – Non-model organism – One Health – Reservoir – Rodent.

Introduction

The human genome project ushered in the modern genomics era, offering enormous promise of providing answers to the fundamental question of what makes us human. All was not revealed, however, when the ~3 billion letters of human DNA were made publicly available. If anything, the situation suddenly became significantly more complex. To find out what was really special required something to compare ourselves to, hence comparative genomics. Starting with small laboratory animals used to model human diseases (e.g. mouse, rat) and close human relatives (e.g. chimpanzee, macaque), the amount of genome sequence data has since grown exponentially. Representatives of diverse taxa have now been sequenced, and a large number of species are in the pipeline to have their genomes sequenced (1). The main factor underlying the meteoric rise in genomic data has been the decrease in the cost of sequencing (2). Initially, sequencing priority was given to species with immediate medical or economic significance. At that time, sequencing more distantly related species had two main purposes: to identify constrained elements within the human genome (what makes us human?); and to reveal

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deep evolutionary relationships among species through genome-wide phylogenomics (where do we fit onto the tree of life?). Divergent and outbred species have since come to be viewed as goldmines of genetic diversity in their own right, leading to a surge in activity to identify genetic elements associated with interesting and unusual, naturally occurring phenotypes. A very recent trend is the sequencing of species that are natural reservoir hosts of pathogens of humans and animals.

As a field of study, 'reservoir host biology' is fast gaining momentum (3, 4). So, why has this interest in natural reservoir hosts arisen now? Severe acute respiratory syndrome (SARS), Nipah virus, Middle East respiratory syndrome (MERS), Ebola, globalisation, international travel, climate change, Hollywood, social media and the threat of bioterrorism have all played a part. Scientifically, this interest stems from the awareness that all newly emerged viral diseases must have come from somewhere in nature. Before and between outbreaks, viruses seemingly disappear, yet as obligate parasites they cannot survive for extended periods outside a living organism. Where do they come from? Where do they go between outbreaks? Where and when might they return? It was long ago recognised that certain diseases such as rabies could be caught directly from wild animals, and the idea of human pathogens circulating in other species is not new, but the concept of wild animals being natural reservoirs of viruses, periodically reintroducing them into human or livestock populations via spillover events, has come a long way in recent times. The crucial observation is that some infectious agents are apathogenic in natural reservoir species but cause aggressive and sometimes lethal infections in other species. The big question is therefore: why do some organisms develop severe or lethal infections, while others are barely affected (if at all), when both are infected with the same virus?

Debate in this area tends to depend upon whether researchers are more virus-centric or more host-centric in their thinking, as the following as-yet-unanswered questions illustrate.

– Do natural reservoir hosts avoid disease by having more effective immunity than spillover hosts?

– Do natural reservoir hosts avoid disease by having less immunopathology than spillover hosts?

– Do natural reservoir hosts avoid disease by having a general mechanism that confers resistance to certain types of disease, for example, cyclic body temperature?

– Do natural reservoir hosts avoid disease because the viruses that infect them have simply evolved to an optimal or equilibrium state? For example, are viruses simply better adapted at controlling their replication rate so that they effectively avoid pathogen detection mechanisms in their native host, provoking neither disease nor sterilising immunity?

– Is the absence of disease during infection the normal state of interaction between different organisms, whereas the development of disease is the anomaly? (Is 'not sick' even a phenotype?)

Such questions expose wide gaps in our knowledge. For example, while the true nature of viral pathogenesis is, in some sense, due to cell death or dysfunction, how do we separate the intertwined roles of virus and cell in this process? Cross-disciplinary terms such as 'host–pathogen interaction' implicitly acknowledge this duality. Most importantly, however, we just do not know enough about the processes involved, and our tentative answers to these questions remain somewhat speculative. If our aim is to understand how human and animal diseases develop, then we need to compare susceptible species to those that do not develop disease, and when it comes to infectious diseases, natural reservoir hosts demonstrate the exact phenotype we seek. Comparative genomics, as it relates to infectious diseases, asks therefore: what exists in the diseased host but not in the asymptomatic one (and vice versa)? Could one be made to behave more like the other through intervention? If any natural virus resistance phenotype can be translated into a medical intervention it will be a truly remarkable feat. In this sense we will have learned from nature.

While 'genomics' specifically refers to the study of the genome of an organism (both nuclear and mitochondrial DNA), the word has come to represent a large range of information types loosely connected by the idea of being 'genome-wide'. The term 'omics' captures the general idea of complete sets of information rather than samples or subsets. While genomes are essentially static over short time spans (except, for example, in cancer), the other 'omics' encompass highly dynamic systems; for example: transcriptomics (coding and non-coding RNA), epigenomics (chromatin modifications), proteomics, glycomics and metabolomics. The options in this field can include a broad list of 'omics' data representing multiple tissues, multiple cell types, multiple states (e.g. infected versus non-infected), multiple time points, multiple viruses and multiple species. Realistically, of course, we can only scratch the surface at this time. The most practical/realistic direction to follow is therefore to keep the focus fairly narrow but to dig deeper, rather than focusing broadly but remaining superficial. A single well-understood case would be much more useful than a large collection of poorly understood cases. Given the very wide spectrum of possibilities involved, there seems to be a risk of continually opening new lines of enquiry at the expense of building upon work that has already been done. Clearly, great challenges still lie ahead.

Genomics of representative natural reservoir hosts

Genomics research into species that are natural reservoir hosts of viruses of humans and domestic animals has had only a short history, but it has already begun to yield insights into the mechanisms of host–pathogen interaction that would have been difficult or impossible to achieve without a genome-wide approach. In this review, the authors focus on four major groups of natural reservoirs: bats, birds, rodents and non-human primates (Fig. 1). At the time of writing, the number of publicly available genome sequences representing these four taxa stands at 10 (bats), 59 (birds), 12 (rodents), and 23 (non-human primates) (Table I).

Bats

Bats are confirmed or suspected natural reservoir hosts of over 200 different viruses from 27 different viral families (41). These include high-profile pathogens such as SARS coronavirus, MERS coronavirus, Ebola virus, Marburg virus, Hendra virus (HeV), Nipah virus (NiV) and rabies viruses. The presence of viral pathogens in apparently healthy, wild-caught bats is consistent with the role of a natural reservoir host, and numerous experimental infection studies have also failed to induce a number of viral diseases in bats. Under certain conditions, however, bats do succumb to infectious diseases. A fungal infection that causes white-nose syndrome has decimated North American bat populations (42), and at least one example of viral disease in a bat has been described recently (43), suggesting that Jamaican fruit bats (*Artibeus jamaicensis*) are not a natural reservoir of Tacaribe virus. Experimental infections of bats with rabies virus have produced varying results, but in some studies bats have been shown to recover naturally from rabies infection without any intervention. Furthermore, healthy bats with antibodies against rabies virus have been identified in the wild, distinguishing them from other mammals amongst which, once established, rabies is usually fatal (44). Accumulating evidence appears to support the conclusion that bats are ancestral hosts of a number of important viral families (45, 46, 47).

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Fig. 1

Significant numbers of bats, birds, rodents and non-human primates are natural carriers of human and animal pathogens

Genomic comparisons between natural reservoir hosts and disease-susceptible spillover hosts can reveal differences that may inform the development of new strategies for treating infectious diseases

Table I
Genome sequences of bats, rodents, non-human primates and birds

Scientific name	Common name	Genome size (Mb)	Coverage	Reference
Bats				
Eidolon helvum	Straw-coloured fruit bat	1,837.75	18×	5
Eptesicus fuscus	Big brown bat	2,026.63	84×	
Megaderma lyra	Indian false vampire	1,735.93	18×	5
Myotis brandtii	Brandt's bat	2,053.02	120×	6
Myotis davidii	Mouse-eared bat	2,059.8	110×	7
Myotis lucifugus	Little brown bat	2,034.58	7×	8
Pteronotus parnellii	Parnell's moustached bat	1,960.31	17×	5
Pteropus alecto	Black flying fox	1,985.96	110×	7
Pteropus vampyrus	Large flying fox	2,198.28	188×	8
Rhinolophus ferrumequinum	Greater horseshoe bat	1,926.43	17×	5
Rodents				
Cavia porcellus	Guinea pig	2,723.2	6.8×	8
Chinchilla lanigera	Long-tailed chinchilla	2,384.46	87×	
Cricetulus griseus	Chinese hamster	2,397.87	130×	9
Dipodomys ordii	Kangaroo rat	2,236.37	181×	8
Heterocephalus glaber	Naked mole rat	2,611.29	90×	10
Ictidomys tridecemlineatus	Thirteen-lined ground squirrel	2,478.39	495×	8
Jaculus jaculus	Lesser Egyptian jerboa	2,826.89	78×	
Mesocricetus auratus	Golden hamster	2,438.24	115×	
Mus musculus	Mouse	2,477.64	ND	11
Nannospalax galili	Upper Galilee Mountains blind mole rat	2,984.2	86×	12
Octodon degus	Degu	2,989.51	80×	
Rattus norvegicus	Rat	2,870.18	ND	13
Non-human primates				
Aotus nancymaae	Ma's night monkey	2,896.49	113×	
Callithrix jacchus	White-tufted-ear marmoset	2,914.96	6.6×	14
Cercocebus atys	Sooty mangabey	2,848.25	192×	
Chlorocebus sabaeus	Green monkey	2,789.66	95×	
Colobus angolensis	Angolan colobus	2,970.12	87×	
Daubentonia madagascariensis	Aye-aye	2,856.38	38×	15
Gorilla gorilla	Western gorilla	3,035.66	ND	16
Macaca fascicularis	Crab-eating macaque	2,946.84	68×	17
Macaca mulatta	Rhesus monkey	3,097.39	ND	18
Macaca nemstrina	Pig-tailed macaque	2,948.7	113×	
Mandrillus leucophaeus	Drill	3,061.99	117×	
Microcebus murinus	Grey mouse lemur	2,438.8	222×	8
Nasalis larvatus	Proboscis monkey	3,011.97	290×	
Nomascus leucogenys	Northern white-cheeked gibbon	2,962.06	ND	19
Otolemur garnettii	Small-eared galago	2,519.72	137×	8
Pan paniscus	Pygmy chimpanzee	2,852.91	26×	20
Pan troglodytes	Chimpanzee	3,309.58	ND	21
Papio anubis	Olive baboon	2,948.4	92×	
Pongo abelii	Sumatran orangutan	3,441.24	ND	22
Propithecus coquereli	Coquerel's sifaka	2,798.14	105×	
Rhinopithecus roxellana	Golden snub-nosed monkey	2,790.58	54×	23
Saimiri boliviensis boliviensis	Bolivian squirrel monkey	2,605.18	80×	
Tarsius syrichta	Philippine tarsier	3,042.57	48×	8
Birds				
Acanthisitta chloris	Rifleman	1,035.88	29×	24
Amazona vittata	Puerto Rican parrot	1,175.40	24×	25
Anas platyrhynchos	Peking duck	1,105.05	60×	26
Anser cygnoides	Swan goose	1,119.13	107×	27

Scientific name	Common name	Genome size (Mb)	Coverage	Reference
Apaloderma vittatum	Bar-tailed trogon	1,070.84	28×	24
Aptenodytes forsteri	Emperor penguin	1,254.35	60×	24
Aquila chrysaetos	Golden eagle	1,548.48	88×	28
Ara macao	Scarlet macaw	1,204.70	26×	29
Balearica regulorum	Grey-crowned crane	1,127.62	33×	24
Buceros rhinoceros	Rhinoceros hornbill	1,065.78	35×	24
Calypte anna	Anna's hummingbird	1,105.68	110×	24
Caprimulgus carolinensis	Chuck-will's-widow	1,119.68	30×	24
Cariama cristata	Red-legged seriema	1,132.25	24×	24
Cathartes aura	Turkey vulture	1,152.57	25×	24
Chaetura pelagica	Chimney swift	1,119.19	106×	24
Charadrius vociferus	Killdeer	1,219.86	100×	24
Chlamydotis macqueenii	MacQueen's bustard	1,086.57	27×	24
Colinus virginianus	Northern bobwhite	1,171.86	77×	30
Colius striatus	Speckled mousebird	1,075.93	27×	24
Columba livia	Pigeon	1,107.99	60×	31
Corvus brachyrhynchos	American crow	1,091.31	80×	24
Corvus cornix	Hooded crow	1,049.96	152×	32
Cuculus canorus	Common cuckoo	1,153.89	100×	24
Egretta garzetta	Little egret	1,206.50	74×	24
Eurypyga helias	Sunbittern	1,088.02	33×	24
Falco cherrug	Saker falcon	1,174.81	147×	33
Falco peregrinus	Peregrine falcon	1,171.97	138×	33
Ficedula albicollis	Collared flycatcher	1,118.34	60×	34
Fulmarus glacialis	Northern fulmar	1,141.40	33×	24
Gallus gallus	Red jungle fowl	1,046.93	12×	35
Gavia stellata	Red-throated loon	1,129.69	33×	24
Geospiza fortis	Medium ground-finch	1,065.29	115×	24
Haliaeetus albicilla	White-tailed eagle	1,133.55	26×	24
Haliaeetus leucocephalus	Bald eagle	1,178.41	103×	24
Leptosomus discolor	Cuckoo roller	1,136.24	32×	24
Manacus vitellinus	Golden-collared manakin	1,145.85	110×	24
Meleagris gallopavo	Turkey	1,128.34	35×	36
Melopsittacus undulatus	Budgerigar	1,117.37	23×	24
Merops nubicus	Carmine bee-eater	1,062.96	37×	24
Mesitornis unicolor	Brown mesite	1,087.29	29×	24
Nestor notabilis	Kea	1,053.56	32×	24
Nipponia nippon	Crested ibis	1,223.86	105×	24
Opisthocomus hoazin	Hoatzin	1,203.71	100×	24
Pelecanus crispus	Dalmatian pelican	1,160.92	34×	24
Phaethon lepturus	White-tailed tropicbird	1,152.96	39×	24
Phalacrocorax carbo	Great cormorant	1,138.97	24×	24
Phoenicopterus ruber	American flamingo	1,132.18	33×	24
Picoides pubescens	Downy woodpecker	1,167.32	105×	24
Podiceps cristatus	Great-crested grebe	1,134.92	30×	24
Pseudopodoces humilis	Tibetan ground-tit	1,043.00	96×	37
Pterocles gutturalis	Yellow-throated sandgrouse	1,069.32	25×	24
Pygoscelis adeliae	Adelie penguin	1,216.62	60×	24
Serinus canaria	Common canary	1,152.10	45×	38
Struthio camelus	Common ostrich	1,225.04	85×	24
Taeniopygia guttata	Zebra finch	1,232.14	5.5×	39
Tauraco erythrolophus	Red-crested turaco	1,155.54	30×	24
Tinamus guttatus	White-throated tinamou	1,047.06	100×	24
Tyto alba	Barn owl	1,120.14	27×	24
Zonotrichia albicollis	White-throated sparrow	1,052.60	63×	40

Mb: megabase
ND: not determined due to chromosome sequencing

Bat immunology has been studied sporadically since the 1960s, but consistent progress has only been possible over the last decade. Bat immunological findings have been recently reviewed and extensively discussed (48, 49). In the last few years, genomics has begun to shed light on the role of bats as natural virus reservoirs. Ten complete bat genomes are currently available (Table I) and sequencing is currently under way for the common vampire bat, *Desmodus rotundus* (1). Bat genomics to date has largely focused on finding features that are not present in other mammalian species. These include gene duplications, transposons, virus-like sequences, insertions, deletions or nonsense mutations. There have also been efforts to identify genes under positive selection. One study examined two complete bat genomes in relation to their role as virus reservoirs (7). A number of immune genes were found to be under positive selection, and one important immune gene family (AIM2) was entirely absent. Furthermore, the diversity of natural killer cell receptors was significantly reduced in *Pteropus* bats. An enigmatic finding was that multiple DNA damage response genes are under positive selection in bats, along with components of the NF-κB pathway. Given the central role of the DNA damage response in cell fate outcomes, and the fact that several viruses are known to interfere with it, this finding raised the prospect that such changes may influence the outcomes of viral infection. Simultaneously, it raised implications with regard to bats' remarkable longevity. This, in turn, raised the possibility that both outcomes may stem from evolutionary adaptations to compensate for the metabolic demands of flight. Metabolic waste products, such as reactive oxygen species, are unavoidable consequences of oxidative metabolism; hence the origin of flight would have placed great strain on bats' ability to maintain genomic integrity. This may, therefore, have led to compensatory adaptations, such as changes to DNA repair systems, which could have conferred other advantages, such as increased life expectancy and the ability to resist genomic parasites, such as viruses. Further evidence of change in the bat DNA damage response pathway includes mutations in genes encoding the tumour suppressor protein p53 and its negative regulator, mouse double minute 2 homolog (MDM2), both of which show disruption of highly conserved nuclear trafficking signals (7). Another group has also raised the prospect of flight playing an important role in bat–virus interaction, since daily body temperature fluctuations between flight and resting states in many ways resemble a fever response (50).

In addition to whole-genome analysis, transcriptomics has contributed significantly to our knowledge and understanding of bat immunity. Transcriptome studies of *Pteropus alecto* (51) and *A. jamaicensis* (52) have yielded detailed information about the structure of bat immune genes and their expression *in vivo*. In a recent study by Glennon *et al.* (53), RNA sequencing (RNA-Seq) was used to examine the transcriptional response of *P. vampyrus* bat kidney (PVK) cells to Newcastle disease virus (NDV), an avian paramyxovirus known to elicit a strong innate immune response in mammalian cells. Analysis of 200–300 differentially regulated genes showed that interferon and antiviral pathways were highly upregulated in NDV-infected PVK cells. Glennon *et al.* then examined the transcription pattern of these genes upon HeV or NiV infection as pteropid bats are known natural reservoirs of henipaviruses (54). In contrast to infection with NDV, HeV and NiV infection of PVK cells failed to induce these innate immune genes (53).

One very unusual outcome of bat genome analysis has been the finding that some bats have significantly high numbers of recently active DNA transposons (55, 56, 57, 58, 59, 60, 61). This seems to be restricted to bats of the vespertilionid lineage, but has important ramifications for the divergence and speciation of this large and highly successful group. Both large and small bats also have significant numbers of unique microRNAs (61, 62). Considering the role that microRNAs play in post-transcriptional gene regulation, it will be very interesting to see what effect they have on viral replication.

A propensity to apoptosis was identified as a putative point of difference between bat and human cells infected with Hendra virus (63). In this study, a method known as proteomics informed by transcriptomics (PIT) (64) used high-throughput sequencing data to construct a *de novo* transcriptome, which was then used to interpret mass spectrometry data acquired from the same samples. This enabled simultaneous genome-wide differential expression analysis of both messenger RNA and proteins. While this technique is currently limited to cell culture, it has proven to be a powerful method even if a reference genome is unavailable, and should therefore find wide application in the study of natural reservoir hosts.

Birds

Birds are natural reservoirs of several human pathogens, including influenza A viruses and West Nile virus. Intriguingly, while some birds are asymptomatic carriers of these viruses, other birds, such as chickens, succumb readily to viral disease. Birds therefore make an excellent case study for comparative genomics of infectious disease. Amongst the avian-borne viruses, strains of highly pathogenic avian influenza (HPAI) are in the highest category of risk for their potential to cause global human pandemics (65). The most virulent HPAI strain is H5N1, which circulates naturally in waterfowl populations. Periodically, HPAI spills over into domestic poultry, where it can be passed on to humans. Chickens are exquisitely sensitive to H5N1 and it is almost uniformly lethal to them, while in humans the mortality rate is about 60%. Wild birds such as ducks are relatively

resistant to disease, consistent with their role as natural reservoir hosts. Susceptible species suffer severe respiratory pathology; however, one factor believed to contribute to the high mortality rate is a deadly systemic inflammatory effect known as a 'cytokine storm'. This apparent role of the immune system in HPAI pathogenesis presents an excellent model for investigating differences between natural and spillover hosts. Indeed, the role of immunopathology in disease progression is a recurrent theme in natural reservoir host biology.

The analysis of avian genomes has revealed a number of differences in immune genes (66). Importantly, chickens lack the pattern recognition receptor RIG-I (67). Recently, the duck genome was compared to the genomes of chickens in the context of avian influenza infection (26). It was shown that, while birds have contracted repertoires of immune genes, such as cytokines, they have expanded repertoires of beta defensins. Ducks were found to have lineage-specific expansion of butyrophilin-like (BTNL) genes that were not observed in either chickens or turkeys. Both defensins and BTNLs were among differentially expressed genes in RNA-Seq analysis of ducks infected with H5N1 strains, and may thus be important mediators of infection outcomes. It has been observed that, unlike chicken cells, duck cells undergo rapid apoptosis when infected with strains of H5N1 to which they are clinically resistant, leading to lower titres of infectious virus (68). A recent study has also suggested that microRNAs may play a role in HPAI pathogenesis (69).

Recently, 48 avian genomes were sequenced by a large international consortium (24), bringing the total number of avian genomes to 59 (Table I). This is now one of the richest datasets in vertebrate genomics, and its potential to shed light on the role of wild birds as natural virus reservoirs is yet to be tested.

Rodents

Rodents have long been associated with human and animal pathogens, particularly after the outbreaks of bubonic plague that ravaged Europe in the Middle Ages. While the true vectors of plague were actually parasitic fleas, rather than the rats upon which they fed, it is nonetheless widely (and correctly) assumed that rodents carry pathogens capable of causing disease. Rodents are considered natural reservoir hosts of several viruses, including members of the genera *Hantavirus* and *Arenavirus*. Given the availability of reagents and other resources for rat and mouse studies, this is potentially an area where discoveries using 'omics' methods might be readily subjected to experimental verification. Twelve rodent genomes are currently available for comparative genomics (Table I). In the absence of genome data for natural reservoirs of hantaviruses, studies have used quantitative real-time polymerase chain reaction (qRT-PCR) arrays (70) and RNA-Seq (71) to measure transcriptional responses in wild rodents. It has been shown that genes involved in regulating T helper follicular cell development may facilitate viral persistence in a natural reservoir of Andes virus (71).

Non-human primates

The image of a wild monkey carrying a deadly human disease is firmly established in our collective imagination. In reality, it is perhaps surprising that, as our closest relatives, primates have been implicated as the source of only a few human infectious diseases. One such disease, however, has caused the largest human pandemic in recent history. Human immunodeficiency virus (HIV) is not spread by contact with wild animals. However, it is believed to have originated in African primates from an ancestral virus closely related to simian immunodeficiency virus (SIV). As a model of human HIV infection, SIV has been extensively studied in non-human primates such as the rhesus macaque, which develops an acquired immunodeficiency syndrome (AIDS) similar to human AIDS. Great interest has surrounded natural reservoirs of SIV, such as the sooty mangabey (*Cercocebus atys*). Although primate reservoir hosts are susceptible to SIV infection, they do not progress to AIDS (72). Microarray analysis has demonstrated that natural reservoir hosts of SIV exhibit a strong inflammatory response during the acute phase of infection, including potent induction of interferon-stimulated genes (ISGs). However, this response rapidly attenuates following peak viral load, unlike in non-natural hosts, where interferon responses are sustained at high levels (73). In humans infected with HIV, high levels of virus replication during the chronic phase typically correlate with rapid disease progression; however, a rare subset of individuals sustain high-level virus replication yet do not progress to immunodeficiency (termed viraemic non-progressors) and exhibit similar low-level ISG induction (74). The sooty mangabey and other primate genomes are available for comparative analysis (Table I).

Challenges in reservoir host genomics

The overarching goal of natural reservoir host genomics is to understand the role of host factors in the development of disease as a consequence of infection. This represents a paradigm shift in infectious disease research. While it is now recognised that large numbers of human pathogens (possibly >70%) originated in wildlife (75), the traditional separation of human and animal studies is only just beginning to erode, coinciding with the momentum of the One Health research movement (76). While, at face value,

the association of wild animals and viruses of humans and domestic animals presents a very compelling case, it is important to consider the large number of species involved. Currently, we recognise > 1,200 different species of bats, > 1,500 species of rodents, and > 10,000 species of birds. Only a fraction of these have been given rigorous scientific attention, and any discussion of natural reservoirs needs to be examined within this context. Furthermore, it must be remembered that the term 'reservoir host' is also a pathogen-specific one. A particular species may act as a natural reservoir for one virus, yet may have no such special relationship with other viruses. Whether bats are 'special' virus reservoirs remains to be seen (77, 78, 79, 80), and more work needs to be done to validate broader connections between bats and emerging viruses (81). Nevertheless, whether bats, birds, rodents or primates turn out to be 'special' or merely 'typical' reservoirs, they are still undoubtedly sources of disease. Comparative genomics offers an opportunity to unravel mechanisms conferring disease resistance and susceptibility, and therefore deserves our serious attention.

While the current wave of genome sequencing appears unstoppable, many researchers are familiar with the unfortunate reality that meaningful biological insights from genome-sized data have, in many cases, not yet materialised. A major issue is that genome-scale data form too big and complex a dataset for many laboratories to manage. This does not indicate a shortcoming in computing power or technical infrastructure, but rather a global shortage in bioinformatics skills. Very few biologists are proficient programmers, while most computer scientists have little more than rudimentary knowledge of molecular biology. The rise of graphical genomics software and cloud computing enables many life scientists to engage in 'omics' research; however, the interpretation of genomics data, particularly in comparative genomics studies, remains a very challenging task. Computing skills (and, more importantly, computational thinking) are gradually becoming more widely appreciated in the life sciences field, and 'wet-lab' researchers are gradually becoming accustomed to working with bioinformaticians, statisticians and other 'dry lab' scientists. Thus, the problem should naturally resolve itself in the near future.

Future perspectives

Future virus surveillance methods will likely incorporate the high-throughput sequencing of samples collected from known and suspected reservoir hosts in the wild, including blood samples and metagenomic material, such as urine, faeces and environmental samples. Proper interpretation of these data will be greatly aided by the availability of reference genomes that enable both the identity and health status of host animals to be evaluated, as well as helping workers to distinguish sequence data of host origin from data that may represent divergent viruses which are otherwise extremely hard to detect. It is already clear that evolutionary insights into host–pathogen interaction can also be informed by sequence data (82).

Genome-wide small interfering RNA (siRNA) libraries and, more recently, CRISPR/Cas9 libraries represent powerful systems for identifying host genes involved in virus replication. The success of this approach depends firmly upon having a well-annotated genome. When such a method is applied to reservoir host species, it will be very informative to determine whether the genes that affect virus replication in reservoir hosts are the same as those that affect virus replication in spillover hosts. Can we use genetic markers identified in reservoir hosts to confer resistance in domestic animals through selective breeding or precision genome engineering? Can we identify host factors such as cytokines that promote disease progression in spillover hosts, and may therefore make suitable drug targets? Can we develop vaccines for wild species, such as bats or birds, to prevent spillover events from occurring in the first place? To what extent will a knowledge of reservoir host immune systems be required to achieve this? Beyond the mechanisms of infectious disease resistance, what other unexpected benefits, such as discoveries relevant to cancer or ageing, may arise though the investigation of natural host genomes? Other potential applications of reservoir host genomics include reagent development, disease forecasting and hypothesis testing in developmental and evolutionary biology.

Biological sciences are in the midst of a disruptive, technology-driven revolution. It is almost impossible to imagine what we will be doing in genomics ten years from now. However, it is likely that we will wish to obtain representative genome sequences of all known species, and, for species of particular interest, we may sequence thousands or even millions of individuals. It is possible that a large proportion of humanity will eventually have their genomes sequenced. This will be further complemented by the whole gamut of 'omics' data. But why continue to do this? Medicine, conservation, evolutionary studies, economic gain? Is there any limit to what we will find worth sequencing? Of course it all depends on whether we continue to make discoveries that can be translated into benefits for humankind. What will we find when we compare millions of species in quantitative ways? It seems possible that even very hard problems might succumb to the flood of data if we ask the right questions. Clearly, genomics alone will not solve the question of what makes an animal resistant or prone to disease, but it will help guide discovery. It is at the forefront of the new biology and yet this would have been almost unimaginable at the dawn of the genomics era, just 15 years ago. Perhaps, in light of that, we should simply learn to expect the unexpected.

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La génomique animale et les réservoirs naturels des agents infectieux

C. Cowled & L.-F. Wang

Résumé

En général, les réservoirs naturels sauvages des virus, qu'il s'agisse de chauves-souris, d'oiseaux, de rongeurs ou de primates non humains ne font pas partie des espèces modèles utilisées pour l'expérimentation et jusqu'à une époque récente il était particulièrement difficile de mener des recherches approfondies sur ces animaux, difficiles d'accès. Le séquençage de nouvelle génération permet de contourner partiellement cette limitation, puisque les méthodes nécessaires pour obtenir et analyser les données de séquences sont en grande partie indépendantes de l'espèce étudiée. La génomique comparée, au même titre que d'autres technologies dites « omiques » offre de nouvelles possibilités d'étudier la structure et les fonctions de systèmes biologiques appartenant à une variété d'espèces sauvages auxquelles les chercheurs n'ont aucun accès. L'étude du génome des hôtes réservoirs naturels permet d'identifier les voies privilégiées d'interaction entre le virus et l'organisme hôte et d'élucider ce qui distingue les espèces, les populations et les organismes sensibles de ceux qui sont résistants. Cet aspect présente un grand intérêt scientifique et pourrait générer les données nécessaires à la conception de traitements rationnels contre les maladies virales humaines ou animales. C'est ainsi que nous « apprendrons de la nature » et pourrons un jour utiliser ces connaissances pour donner naissance à des animaux d'élevage résistants aux maladies ou pour mettre au point des stratégies thérapeutiques et prophylactiques innovantes. D'autres perspectives prometteuses de la génomique des hôtes réservoirs portent sur la mise au point de vaccins innovants destinés à la faune sauvage et sur le développement de nouvelles plateformes diagnostiques. La génomique aura également un impact important en biologie du développement et de l'évolution. La synthèse présentée par les auteurs est axée sur les hôtes réservoirs naturels des virus, mais la plupart des points examinés s'appliquent tout aussi bien aux réservoirs naturels d'autres agents pathogènes tels que les bactéries, les champignons ou les parasites.

Mots-clés

Chauve-souris – Génomique – Oiseau – Organisme non utilisé comme modèle – Primate non humain – Réservoir – Rongeur – Séquençage de nouvelle génération – Une seule santé.

Genómica de los reservorios naturales de enfermedades infecciosas

C. Cowled & L.-F. Wang

Resumen

En general los animales salvajes que sirven de reservorios naturales de virus, tales como murciélagos, aves, roedores o primates no humanos, no constituyen «organismos modelo» para la ciencia, por lo que hasta hace poco ofrecían escasas oportunidades de estudio detallado. La secuenciación de próxima generación proporciona un medio para salvar parcialmente este obstáculo, por cuanto los métodos requeridos para obtener y analizar datos son, en gran medida, independientes de la especie de que se trate. La genómica comparada y otras técnicas «ómicas» abren nuevas posibilidades para estudiar la estructura y función de varios sistemas biológicos de especies salvajes que de otro modo quedarían fuera de nuestro alcance. El genoma de especies que constituyen reservorios naturales puede ser útil para determinar las vías predominantes de interacción virus–anfitrión y revelar diferencias entre los organismos (o poblaciones o especies) sensibles y los resistentes. Ello, además del gran interés científico que reviste, puede suponer un recurso para la concepción racional de terapias contra enfermedades víricas del hombre y el ganado. De esta manera «aprenderemos de la naturaleza» y algún día emplearemos este saber para generar ganado resistente a determinadas enfermedades o poner a punto nuevos métodos de tratamiento o prevención. La genómica de los reservorios también abrirá vías para obtener nuevas vacunas destinadas a los animales salvajes, ayudará a crear nuevos dispositivos de diagnóstico y tendrá repercusiones de gran calado para la biología evolutiva y del desarrollo. Los autores se centran aquí en los reservorios de patógenos víricos, aunque la mayoría de los aspectos examinados deberían poder aplicarse igualmente a los reservorios naturales de patógenos bacterianos y fúngicos u otros parásitos.

Palabras clave

Aves – Genómica – Murciélagos – Primate no humano – Organismo «no modelo»– Reservorio – Roedor – Secuenciación de próxima generación – Una sola salud.

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