provide a means for cellular growth.

Under nutrient deprivation, diploid *S. cerevisiae* could adopt three fates: spore formation, quiescence (when cells do not divide), or pseudohyphal growth (12). Glucose, when available, is completely fermented to ethanol and acetate, which are further metabolized by respiration upon diauxic shift from the fermentative to the oxidative metabolic state. In late stages of growth, particularly in the center of colonies where nutrients are depleted, and nonfermentable carbons (such as acetate) accumulate, yeast cells could undergo quiescence. Alternatively, ammonia could stimulate the dimorphic switch from ovoid to pseudohyphal cells at the edge of a colony and allow *S. cerevisiae* to extend out and scavenge in the surrounding environment (12).

Spinelli et al. report that, similar to yeast cells in colonies, ER+ breast cancer cells could forage ammonia and fix nitrogen to produce biomass. Specifically, ER+ breast cancer cells have elevated expression of GS and GDH compared to other subtypes of breast cancer. GDH is elevated in luminal type (mostly ER+) breast cancers and associated with quiescent cells in organoid cultures of breast epithelial cells, whereas proliferative cells were dependent on transaminases (13). Transaminases transfer nitrogen (in an amino group) from glutamate to either pyruvate or oxaloacetate to produce α-ketoglutarate and the amino acids alanine or aspartate, respectively. α-Ketoglutarate is further catabolized in the tricarboxylic acid cycle. Spinelli et al. documented through 15N-labeling followed by mass spectrometry the assimilation of nitrogen into the amino acids glutamate, aspartate, proline, or leucine in a manner that required nitrogen fixation and conversion of α-ketoglutarate to glutamate by GDH. They show that GDH is required for ammonia assimilation and that ammonia in situ could accumulate in sufficient amounts in vivo for assimilation via reductive amination of α-ketoglutarate. Furthermore, GDH is required for ER+ breast cancer cell growth in vitro and in vivo. This study underscores the frenzied feeding of cancer cells to survive, but the scavenging phenotypes are dependent on contextual differences between luminal breast cancers (which are ER+) versus basal breast cancers, which do not express ER and which do not exhibit nitrogen fixation (13).

Given the previous observation that GDH is associated with quiescent breast cancer cells in three-dimensional cultures, further studies are necessary to determine whether there is intratumoral heterogeneity in nitrogen fixation—for example, whether well-perfused areas of the tumor produce ammonia that can then be utilized by more poorly supplied areas, reminiscent of the behavior of yeast in colonies.

The metabolic TME produced by resident cells, such as fibroblasts and macrophages, can create an immunosuppressive environment. For example, the metabolites lactate, kynurenine, and adenosine in the TME have been shown to be immunosuppressive (14). Hence, it will be of great interest to further understand whether products such as ammonia could affect tumor immunity or induce autophagy (a process of cellular recycling for biosynthesis) in specific cells (15).

Our richer understanding of cancer metabolism could provide new therapeutic strategies, but the manipulation of metabolic pathways for cancer therapy requires deeper understanding of how metabolic inhibitors affect the components of the TME. It is hoped that a strategy will emerge that could diminish tumor cell survival while promoting an immune-permissive environment.

**REFERENCES AND NOTES**


**ACKNOWLEDGMENTS**

Our work is supported by National Cancer Institute grants CA053497 and CA057341, and the Ludwig Institute for Cancer Research. I thank members of my laboratory for their comments.

10.1126/science.aal070

**MICROBIOLOGY**

**Evolution of neurovirulent Zika virus**

A Zika virus mutation leads to increased neurovirulence

By Gavin Screaton* and Juthathip Mongkolsapaya*

In 2015, Zika virus (ZIKV) became headline news after its association with fetal microcephaly (severely reduced head circumference) in Brazil and was declared a public health emergency by the World Health Organization (WHO) (1). However, ZIKV was not new, it was first isolated from the Zika forest, Uganda in 1947 (2). ZIKV incited little interest compared to other flaviviruses, such as dengue virus (DENV), as it was not thought to cause severe disease. ZIKV infections were largely sporadic, and symptoms were usually mild and flu-like, with self-limiting fever, rash, and conjunctivitis. Around 80% of cases were asymptomatic, and epidemic activity had not been described (3). In 2007, large-scale explosive outbreaks of ZIKV infection were described in Micronesia, and the virus spread across the Pacific, reaching South America in 2015, where it rapidly spread through Brazil and neighboring countries (3–5). On page 933 of this issue, Yuan et al. (6) compared sequences of contemporary ZIKV strains with ancestral ZIKV isolates and describe a mutation that increases the neurovirulence of contemporary strains, which they propose underscores the increased pathogenicity of recent outbreaks.

During these explosive outbreaks, more severe sequelae of ZIKV infection were recognized, including Guillain-Barré syndrome, an immune-mediated demyelinating motor and sensory peripheral neuropathy leading to paralysis, which was first described in the French Polynesian ZIKV outbreak. A major cluster of microcephaly cases was recognized in northeastern Brazil in late 2015 (7, 8). ZIKV was subsequently isolated from the brain of a microcephalic fetus, and the connection between ZIKV infection during pregnancy and a variety of fetal neurological abnormalities

1*Medical Sciences Division, University of Oxford, Level 3, John Radcliffe Hospital, Oxford OX3 9DU, UK. Department of Medicine, Imperial College London, Commonwealth Building, Hammersmith Campus, Du Cane Road, London W12 0NN, UK. Email: Gavin.screaton@imperial.ac.uk; j.mongkolsapaya@ imperial.ac.uk
was recognized (9). A great deal of research has been performed on ZIKV over the past 2 years, including the establishment of animal models of fetal infection. It is now clear that the virus can infect the placenta, causing intrauterine growth retardation, and can passage to the fetus. In fetal brain, ZIKV can infect neural progenitor cells, causing cell death and inhibition of differentiation, leading to cortical thinning and microcephaly (9). Estimates of the risk of neurological damage to the fetus if the mother is infected during pregnancy vary from 1 to 20% and appear to be greatest when the mother is infected during the first trimester (10, 11).

Two big questions that remain about the recent ZIKV outbreak are, why was it so explosive and how had the virus gained the ability to cause neurological damage? One possibility is that the virus has mutated to acquire neurovirulence and the ability to sustain epidemic activity. Yuan et al. looked for such mutations by comparing three contemporary ZIKV strains isolated from patients infected in Samoa, Venezuela, and Martinique in 2015 and 2016, with an ancestral Asian ZIKV strain isolated from a patient in Cambodia in 2010 (6). The three contemporary ZIKV strains were more neurovirulent when injected into 1-day-old mouse brains compared to the ancestral strain (causing 100 versus 16.7% mortality, respectively). In addition, the contemporary strains caused more severe microcephaly when injected into fetal brain and disturbed differentiation and proliferation of cultured neural progenitor cells.

Sequence comparison of the contemporary and ancestral strains revealed that a number of changes had occurred in the contemporary strains. To analyze which of these were virulence-determining mutations, each was introduced into the Cambodian ancestral ZIKV strain and tested in neonatal mouse brain. The Ser<sup>α</sup>-to-Asn<sup>α</sup> (S139N) mutation in the ZIKV polyprotein induced the greatest neurovirulence. This mutation maps to residue 17 of the precursor membrane protein (prM), which, from inference from DENV structures, is likely to be exposed on the surface (see the figure). prM closely associates with envelope protein (E) in flaviviruses and is believed, in part, to act as a “chaperone,” preventing premature fusion of immature virions inside infected cells. In immature virions, 180 copies of E associate with 180 copies of prM, which are arranged into 60 heterohexameric (trimer) spikes. As the immature virion passes through the Golgi apparatus of the host cell, low pH induces conformational change in E, from trimeric to dimeric conformation, potentially exposing the hydrophobic fusion loops of E on the virion surface. However, prM sits on top of the E-fusion loop, preventing association with host cell membranes until it is cleaved by furin protease. prM remains associated with the virus until it falls away after exit from the host cell, leaving mature virions lacking prM and with E arranged in 90 head-to-tail dimers (12).

In DENV, prM cleavage is often incomplete, leading to the production of a range of “partially mature” virus particles containing varying amounts of uncleaved prM. This alters the architecture of the virion surface and has implications for antibody binding, flexibility (or “breathing”) of the virus, and infectivity; virions containing high amounts of prM are noninfectious. It is unknown how “leaky” the cleavage of prM is in ZIKV and therefore how much, if any, uncleaved prM is present, although it is thought that ZIKV virions are more rigid than DENV virions (13).

The S139N mutation in contemporary ZIKV strains is distant from the prM cleavage site, but it remains possible that it may influence cleavage or in some way alter virus stability or flexibility. Although prM has not been formally considered a viral receptor for cell adhesion, it is possible that the S139N mutation creates a new receptor for neural progenitor cells. There is some precedent for prM mediating flavivirus adhesion: In the absence of N-linked glycans on E, the N-linked glycans on prM can mediate West Nile virus infection (14). The level of prM cleavage in ZIKV may have a bearing on this process, and the possibility of mutant prM producing a receptor for neural progenitor cell tropism deserves further investigation.

Another important question remains unanswered—why has there not been an explosive outbreak of ZIKV and associated microcephaly in Southeast Asia? ZIKV has been present in Southeast Asia for several years, with small local outbreaks and occasional cases reported in returning travelers (15). However, because of the similarity to DENV symptoms and the difficulty in distinguishing ZIKV from DENV serologically, the true scale of infection in the region is not clear and may have been underestimated. If this is the case, it is possible that herd immunity has prevented explosive outbreaks, and, in the absence of such peaks in infection, the association with microcephaly has not been unmasked. Robust serodiagnoses to distinguish DENV and ZIKV are needed to further understand these issues.

The association between the S139N change and neurovirulence is tantalizing, but we are far from understanding the mechanism, and it deserves further investigation. In the meantime, the design of ZIKV vaccines has progressed at pace, with several promising candidates entering phase 2 clinical trials. As the incidence of new ZIKV infection has thankfully dropped in the Americas, it will be a challenge to design clinical trials to demonstrate efficacy.

REFERENCES AND NOTES


ACKNOWLEDGMENTS

The authors acknowledge support from Wellcome Trust and the Medical Research Council, UK. G.S. is a Wellcome Trust Senior Investigator.
Evolution of neurovirulent Zika virus
Gavin Screaton and Juthathip Mongkolsapaya

Science 358 (6365), 863-864.
DOI: 10.1126/science.aaq1297