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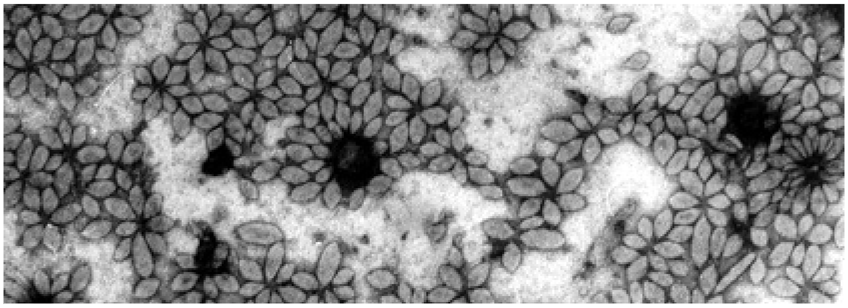


Prospecting for Viruses

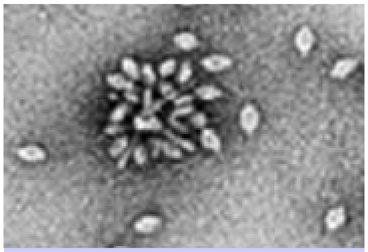
Anne M. Rosenthal

Scientists are prospecting for viruses in the hot springs of Yellowstone National Park - and they are being richly rewarded with intriguing new finds.

The pools where virologists collect typically reach temperatures of 78 to 90 degrees Celsius (172 to 194 degrees Fahrenheit). It's a tough environment for life - unless, that is, you're a thermophile, a life form specially evolved to survive at high temperatures.

Not only are the waters twice the temperature of a comfortable bath, they have an acidity considerably above the survival level of most aquatic organisms. Generally the pools are between pH 2 and 3.5 (between that of lemon juice and carbonated drinks), with some pools as acidic as pH 1 (about the acidity of stomach acid).

Under scalding, acidic conditions, scientists wonder, how do life processes function? Because of their simplicity relative to cellular life forms, viruses may offer scientists the best opportunity to glean information about survival in extreme environments. Viruses are just nucleic acid - either RNA or DNA - protected by a protein coat.


(Click for larger image.) Transmission electron micrographs of virus and virus-like particles isolated from YNP.
Image Credit: Montana State University

Parasites at the molecular scale, viruses take over the machinery of host cells, using it to reproduce themselves. Therefore, the number of genes encoded by viral nucleic acid, generally on the order of 5 to 200, is relatively low in comparison to the thousands typical of cellular organisms. A viral genome usually codes only for the subunits of its protein coat, plus some enzymes - molecules that facilitate chemical reactions, such as nucleic acid replication.

Results from the first studies of thermal viruses in Yellowstone were presented by Mark Young, Co-Director of the Thermal Biology Institute at Montana State University, Bozeman, and Ken Stedman, Assistant Professor of Biology, Portland State University, at the Second Astrobiology Science Conference in Mountain View, CA.

"We're trying to use these viruses as models for understanding biochemical adaptations at high temperatures," states Young.

Clues provided by this new research could give insight on how life existed in the extreme environments present early in Earth's history, or that exist today on other planets. It also could open a window into the biology of the viral hosts, in this case a one-celled thermophile called Sulfolobus, which shares important biological traits with more-complex organisms.

Importantly, the DNA of heat-loving viruses may code for previously unknown enzymes that work efficiently at high temperatures, potential new workhorses for molecular biology laboratories. The tasks of these laboratories range from developing vaccines to unraveling the evolution of life on Earth.

Viral Diversity

A key element of studies by Young, Stedman, and others is viral biodiversity. The scientists are examining the variations in both the morphology (physical structure) and the genomes of viruses from thermal environments.

Because viruses carry just a small number of genes, which code for only a small number of proteins, their coats must be composed of only a few types of protein subunits. Once the subunits are manufactured by the parasitized cell, they self-assemble into the viral coat, also known as the "capsid."

The vast majority of the approximately 3500 described viruses belong to two general morphologies: they are either rod-shaped or have a quasi-spherical shape termed an icosahedron. Similar to a miniature soccer ball, the icosahedron is composed of 5-sided and 6-sided faces (pentamers and hexamers).

But in the thermal hot-spring environment, scientists have found viruses with capsids unlike any previously discovered: Some have one of the traditional shapes but bear unusual structures, while others sport completely novel coat morphologies.

An exquisite iscosahedral virus bearing extensions akin to propellers is one of the intriguing finds. The propeller-like structures have "never been seen before on any kind of virus," says Young.

There are 12 of these propellers on each viral capsid, extruding from the 12 pentamers capping the vertices (corners) of the icosahedron. Like the pentamers in which they are lodged, the propellers have a 5-sided symmetry; the "blades" they bear number five. The pentameric subunits are surrounded by typical viral hexamers that lack special structures.

Extreme Life Briefing

- Hottest: 235 F (113 C) *Pyrolobus fumarii* (Volcano Island, Italy)
- Coldest: 5 F (-15 C) *Cryptoendoliths* (Antarctica)
- Highest Radiation: (5 MRad, or 5000x what kills humans) *Deinococcus radiodurans*
- Deepest: 3.2 km underground
- Acid: pH 0.0 (most life is at least factor of 100,000 less acidic) pH 5-8
- Basic: pH 12.8 (most life is at least factor of 1000 less basic) pH 5-8
- Longest in space: 6 years *Bacillus subtilis* (NASA satellite)
- High Pressure (1200 times atmospheric)
- Saltiest: 30% salt, or 9 times human blood saltiness. *Haloarcula*
- Smallest: <0.1 micron or 500 fit across a human hair width (picoplankton)

Credit: USGS

This virus was originally isolated by Stedman, and the complete structure was determined by Liang Tang, a member of Jack Johnson's group at the Scripps Research Institute in La Jolla, California. In puzzling over the role of the unique propeller structures, scientists keep in mind the steps involved in viral reproduction. First, a virus must be able to dock on the outside of an appropriate host cell, and then, in a second step, release its nucleic acid into the cell's interior.

"We presume that the structures are involved in virus attachment to the host cell or movement of genetic material," says Young. "The density of these structures extends part way into the interior, suggesting that they may be a portal for nucleic acids." Young notes that the propeller-like structures could also be involved with helping newly replicated viruses exit the host cell.

An interesting question is whether or not the structures are dynamic. One possibility, says Stedman, is that the proteins could change conformation, or shape. In so doing, the structures themselves might open, like a door. Alternatively, they could serve as a doorknobs or keys, somehow opening a second structure on the virus or on the host cell.

The virus bearing propeller-like structures, as well as the other interesting viral forms being discovered in Yellowstone Park, are hosted by an organism called Sulfolobus. A member of the kingdom Archaea, which contains single-celled organisms present in many extreme environments, Sulfolobus is found where volcanic activity is present, in hot springs located as far apart as Kamchatka, Italy, Iceland and Yellowstone National Park.

Sulfolobus, a hyperthermophile (an organism that prefers extremely high temperatures), is favored by investigators searching for new hot-spring viruses because it is easy to culture in the laboratory. Explains Stedman, " it is probably the easiest extreme thermophile to work with, since it grows in [the presence of] air." Many other extreme thermophiles are poisoned by oxygen, he notes.

The work on Yellowstone viruses builds on earlier studies by Wolfram Zillig of the Max Planck Institute for Biochemistry, Martinsried, Germany and his research group. Their work identified a number of new viruses in Sulfolobus. So different were the Sulfolobus finds from previously described viruses, that the new viruses were placed in four new viral families.

Finding four new viral families living within a single organism was unprecedented, Young says. Prior to the studies on Sulfolobus viruses, the approximately 4000 described viruses were categorized into about 75 families. Few new family additions had been made for perhaps twenty years. Even studies of viruses found in other members of the Archaea, such as salt-loving and methane-producing species, had not yielded new families of viruses.

In all, Young and Stedman have discovered ten novel viruses, never described before, hosted by Yellowstone Park Sulfolobus. These include the "propeller virus" as well as a virus-like particle, lemon-shaped with long appendages at each end. This particle is so large - 5 to 10 times larger than the similarly shaped SSV viruses, found in Sulfolobus by the Zillig lab - that it may not be a virus at all, Stedman notes, but a nano-sized microbe that lives within the larger Sulfolobus as a symbiont.

"It's remarkable," concludes Young. "Every time we look [in Sulfolobus], we find another virus that we are fairly confident is going to be a brand new group." With new discoveries occurring so rapidly, the scientists are just beginning to characterize these finds.

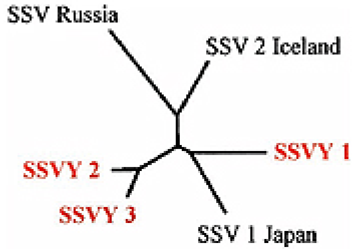
Of great significance is the uniqueness of the viral DNA sequenced so far. "When we sequence these viruses in our labs, we use computer programs that search all public gene banks in the world. The computer programs search billions of sequences and try to align our viral sequence to all other known sequences," explains Young. The thermophilic viruses from Yellowstone have genes with less than 14 percent correlation with known sequences, he notes, " meaning that their genes are not related to other known genes or proteins." This probably reflects the unique biochemical environment where these viruses are found, Young says.

So far most of the viruses found in Sulfolobus are non-lytic: Once reproduced, they reside within the Sulfolobus cells and slip out without destroying their host. Their non-lytic character allows the viruses to remain inside the modulated cellular environment of the Sulfolobus cells; it limits the time the viruses are exposed to the acidic environment outside. "Young's work is really pioneering," says Baruch Blumberg, Director of the NASA Astrobiology Institute. "To date there has not been a great deal of investigation of viruses in the extreme environments. However, there have been sufficient studies to realize that viruses are extremely common, particularly phages of the Archaea and the Bacteria."

Viruses Provide Clues

Sulfolobus is an important organism to study because it is an extreme thermophile - that is, it grows at extremely high temperatures, explains Stedman. Such a archaeans use proteins similar to those in more-complex cells, such as human cells, for certain important biochemical processes involving nucleic acids. But the Sulfolobus proteins are much simpler, making them easier to study, and thus potential stepping stones to understanding similar proteins in more complex organisms. The Sulfolobus viruses are important tools for understanding the machinery of the heat-loving archaeans that host them, adds Stedman.

Importantly, "the complete genome sequence [of Sulfolobus] has been determined, so we know exactly what DNA is present in the organism," Stedman says. But, he cautions, "We don't know what it does." In other words, explains Stedman, they have the blueprint but have not studied most of the proteins it codes for. This also "is true of the human genome, but the [Sulfolobus] blueprint I have to work with is 1000 times smaller than the human blueprint," Stedman explains. The smaller size makes the Sulfolobus genomic blueprint a good starting point; the viruses parasitizing Sulfulobus are potential tools for studying this blueprint.



Dendrogram from sequence alignment of SSV VP3 protein sequences [Phylip (19); Biology WorkBench].
Image Credit: Montana State University

Blumberg, who won a Nobel prize for his work on the hepatitis B virus, comments that, as a viral researcher for many decades, he was "aware of the profound effect that the hepatitis virus has on the liver cell. We've learned a lot about the liver cell by understanding how the virus worked."

One way that Stedman is studying Sulfolobus is with a shuttle vector he created using the SSV1 virus.

"A shuttle vector is a piece of DNA which can replicate in two different organisms - in this case the bacterium E. coli and [the archaean] Sulfolobus - i.e., something that one can use to move or 'shuttle' DNA from one organism to another. This is extremely useful for the development of molecular genetics," explains Stedman.

"The significance of the shuttle vector is three-fold. First, it allows researchers to introduce genes into Sulfolobus to study how they function in the cell," says Stedman.

"Secondly," he adds," it allows the purification of large amounts of viral DNA from E. coli, the workhorse of modern molecular biology." In other words, the shuttle vector allows scientists to produce the DNA of viruses that normally grow in the archaean Sulfolobus in the bacterium E. Coli instead.

"Thirdly," concludes Stedman," it allows much more flexibility in the study of the virus, since modifications of the viral genome can be made in the lab in E. coli and then re-introduced into Sulfolobus.

What's Next

Young, Stedman, and their colleagues plan to look for additional viral forms in Sulfolobus and other heat-loving archaeans at new locations within Yellowstone. Their recently completed studies looked at viruses from only eight locales, a tiny fraction of the approximately 10,000 thermal features of the national park.

Stedman has begun sampling in Lassen National Park, and Young, along with Zillig and others, have begun looking at the water-saturated soils underlying geothermally heated lakes and mud holes for new viruses. These habitats are extremely hot, often over 100 C (212 F), and host extreme heat-loving organisms, including, in the deeper anaerobic sediments, those that live without oxygen.

Related Web Pages

- Thermal Biology Institute, Montana State University
- Mark J. Young, Ph.D., Co-Director, Thermal Biology Institute
- Ken Stedman, Ph.D., Portland State University
- Virus Micrographs (accessible through Ken's web page) Note regarding Virus Micrographs: Where lemon-like virus particles appear clustered, like the petals on a flower, the virus particles are actually attached to a small fragment of a Sulfolobus bacterium.
- Viruses from Extreme Thermal Environments

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