Final Exam

MB 451
Microbial Diversity

Honor pledge: *"I have neither given nor received unauthorized aid on this test."*

Signed : _____ Date : _____

Name : ___*Key*_____

1. What are the three primary evolutionary branches of life? (5 points)

Bacteria, Archaea & Eukarya

Multiple choice (2 points each, 20 points total)

2. __*D*__ Obligate parasites typically have genomes that are _____ than their free-living relatives.
   A. more G+C-rich
   B. denser
   C. more fragmented
   D. smaller
   E. not much different

3. __*E*__ Difficulties associated with life at high temperatures do ***not*** include...
   A. increased membrane fluidity
   B. nucleic acid denaturation
   C. protein denaturation
   D. small molecule decomposition
   E. all of the above are true

4. __*C*__ The use of two photosystems by cyanobacteria allow them to produce _____ from light?
   A. ATP (energy)
   B. NADPH (reducing power for carbon fixation)
   C. both ATP and NADPH
   D. H2S (for reverse electron flow)
   E. ATP, NADPH and H2S

5. __*A*__ The fundamental difference between the Gram-negative and Gram-positive cell envelop is ...
   A. the presence or absence of an outer membrane
   B. the thickness of the cell wall
   C. whether or not iodine fixes dye to the cell wall
   D. whether they are pink or purple
   E. all of the above

6. __*D*__ Which of the following is ***not*** true?
   A. sulfur respiration = Sulfur + organics --> CO2 + H2S
   B. sulfur oxidation = Sulfur + O2 --> H2SO4
   C. sulfur reduction = Sulfur + H2 --> H2S + protons
   D. all of the above are true
   E. none of the above are true

7. __*B*__ The difference between a eukaryotic nucleus and the nuclear body of *Gemmata* is ...
   A. eukaryotes have a 2-layer thick nuclear envelop
   B. eukaryotic nucleii have few or no ribosomes
   C. eukaryotic nucleii contain chromosomes
   D. transcription inside and translation by ribosomes outside the "nucleus" are unlinked
   E. all of the above

8. __E__ *Caulobacter* is a good system to study gene localization in the nucleoid because...

A. you can isolate synchronously growing populations

B. rounds of DNA replication do not overlap

C. both mother and daughter cells are polarized

D. it can be genetically manipulated easily

E. all of the above

9. __A__ Secondary metabolites are ***not*** ...

A. required for cell growth

B. produced during stationary phase

C. often secreted into the environment

D. produced by nonfilamentous Bacteria

E. used for improved fitness or competitiveness

10. __D__ Which form of motility only works across the surface of a solid substrate?

A. gas vacuoles

B. flagella

C. gliding

D. twitching

E. spirochaete

11. __B__ Viruses differ from obligately-intracellular bacterial parasites because...

A. viruses lack genes for transcription & translation

B. viruses "merge" into the cytoplasm of the host

C. viruses are metabolically inactive outside the host

D. viruses are smaller and have smaller genomes

E. all of the above

Fill in the blank (1 point per blank, 20 points total).

12. List 2 genera from each of these phylogenetic groups of microbes. Fill in 20 blanks - leave 2 blanks empty. IF YOU FILL IN MORE THAN 20 BLANKS, ONLY THE FIRST 20 WILL BE GRADED. There is a list of genera from the notes on the last page of this exam for your reference.

Chlamydia & relatives	*Chlamydia*	*Chlamydiophila*
Thermotoga & relatives	*Thermotoga*	*Thermosipho*
Cyanobacteria	*Prochloron*	*Anabaena*
Firmicutes (low G+C Gram+)	*Bacillus*	*Clostridium*
Actinobacteria (high G+C Gram+)	*Streptomyces*	*Arthrobacter*
Planctomycetes	*Planctomyces*	*Isosphaera*
alpha proteobacteria	*Agrobacterium*	*Rhodomicrobium*
delta proteobacteria	*Myxococcus*	*Desulfovibrio*
epsilon proteobacteria	*Helicobacter*	*Camylobacter*
Euryarchaea	*Methanbacterium*	*Halobacterium*
Crenarchaea	*Sulfolobus*	*Pyrobaculum*

(Many other answers are possible)

Short answer. (points as indicated, 25 points total)

13. Describe in one sentence the most convincing reason that you either *believe* or *don't believe* the Prion hypothesis. (4 points)

I don't buy the Prion hypothesis because the form (strain) of the disease ought to depend on the genetics of the host rather than the form of the disease of the source.

(Many other answers are possible)

Briefly describe one organism in the phylogenetic group indicated. There is a list of genera from the lecture notes on the last page of this exam for your reference. (3 points each)

14. Phylogenetic group : *Thermotoga* & relatives

Name (genus) : *Thermotoga*

Morphology : *Rod with a loose outer membrane*

Energy source : *Organics*

Carbon source : *Organics*

Habitat : *Solfataras & hydrothermal sediments*

Something else about it : *For a long time, this was the most thermophilic bacterium known*

15. Phylogenetic group : Cyanobacteria

Name (genus) : *Prochloron*

Morphology : *Oval*

Energy source : *Light*

Carbon source : *CO2*

Habitat : *Ocean surface water*

Something else about it : *This organism has the same types of photopigments as green plants and algae*

16. Phylogenetic group : Firmicutes

Name (genus) : *Bacillus*

Morphology : *rods, often in filaments*

Energy source : *Organics*

Carbon source : *Organics*

Habitat : *Soil, insects*

Something else about it : *This organism produces endospores*

17 . Phylogenetic group : Crenarchaea

Name (genus) : *Ignicoccus*

Morphology : *Cocci*

Energy source : *Sulfur reduction*

Carbon source : *CO2*

Habitat : *Hydrothermal vents*

Something else about it : *Parasitized by Nanoarchaeum*

18 . Phylogenetic group : Euryarchaea

Name (genus) : *Haloarculum*

Morphology : *Flat squares*

Energy source : *organics (aerobically) or light (anaerobically)*

Carbon source : *Organics*

Habitat : *Salt lakes*

Something else about it : *Has a very high internal salt concentration - ca. 5M potassium glutamate*

19 . Phylogenetic group : Planctomycetes

Name (genus) : *Pirellula*

Morphology : *Stalked ovals, forms rosettes*

Energy source : *Organics*

Carbon source : *Organics*

Habitat : *Freshwater*

Something else about it : *Has an internal cellular membrane (ICM) separating ribosomes & nucleoid containing cytoplasm from the rest of the cytoplasm*

20 . Phylogenetic group : Alpha proteobacteria

Name (genus) : *Rhodomicrobium*

Morphology : *Stalked rods or ovals*

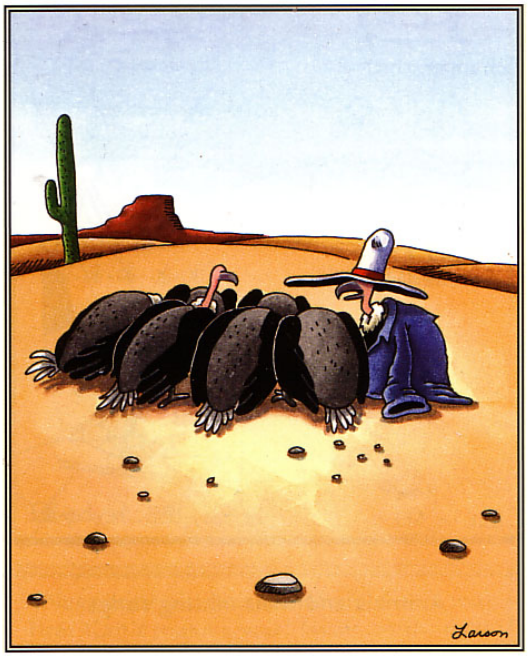
Energy source : *Light*

Carbon source : *Organics (usually) or CO2*

Habitat : *Anaerobic freshwater environments*

Something else about it : *The stalks are cytoplasmic extensions, surrounded by the cell envelop.*

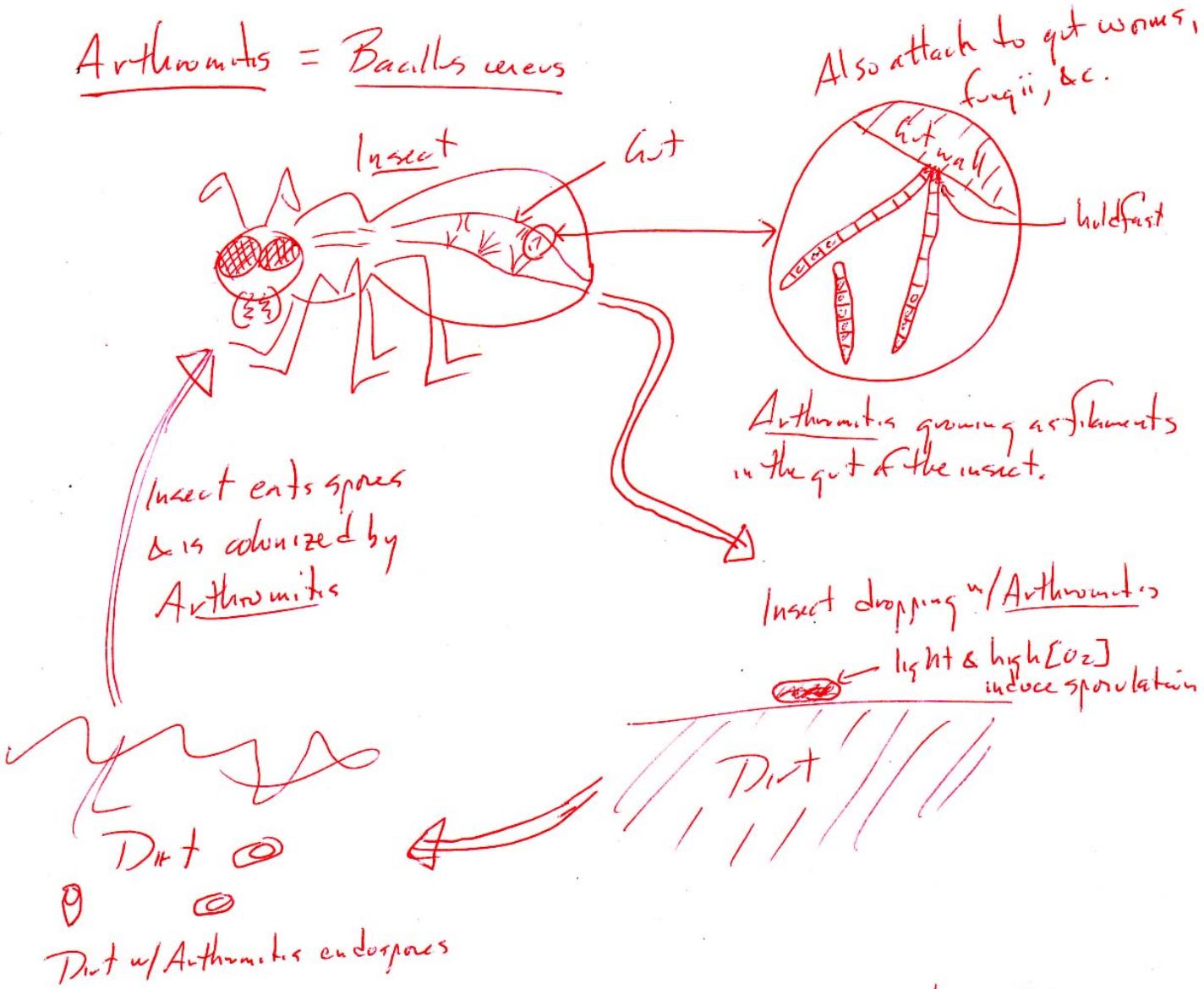
(Many other answers are possible)



"Hey! Look at me, everybody! I'm a cowboy! … Howdy, howdy, howdy!"

Essay (10 points each)

21. **Either** describe in *detail* the life cycle of *Arthromitis*, *Streptomyces*, or *Myxococcus*, **or** summarize in *brief* the life cycle of any of these &/or *Chlamydia*, *Caulobacter*, or *Bdellovibrio*. Be sure to include a diagrams (or diagrams) either way.



(Many other answers are possible)

22. **Either** describe *one* of the papers discussed in class *in detail*, **or** summarize *two* of these papers *in brief*. Either way, be sure to include the question/problem, approach, results and conclusion of the paper(s). A list of these papers is on the last page of this exam for your reference.

Huber H, Hohn MJ, Rachel R, Fuchs T Wimmer VC and Stetter KO. 2002 A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417:63-67

Purpose : This is a monogram, describing this novel organism.

Approach & Results : While isolating organisms from heated underwater gravel off the coast of Iceland, they isolated Igniococcus. They saw that in some of their cultures, the Igniococcus had tiny cocci covering them. In nicely growing cultures, these tiny cocci were nearly all stuck to one of the large Igniococcus cells, but in stationary phase cultures, there were lots of them floating free. These tiny cells could be physically separated by either optical tweezers or standard "sterilizing" filters with a pore size of 0.45um. They could be stained with DAPI, which means they contained DNA, but could never be cultivated in the absence of Igniococcus (the Igniococcus could be grow separately from the small cocci, however). A pure co-culture was obtained by using optical twezers to pick out an Igniococcus cell with a single one of these small cocci attached.

In EM pictures, the small cocci were about 0.4um in diameter, and although they were smashed up against the Igniococcus cells, there were no spacific attachment structures. They looked inside and out like normal Archaea. The small cells could be readily removed from their apparent host by mild sonication.

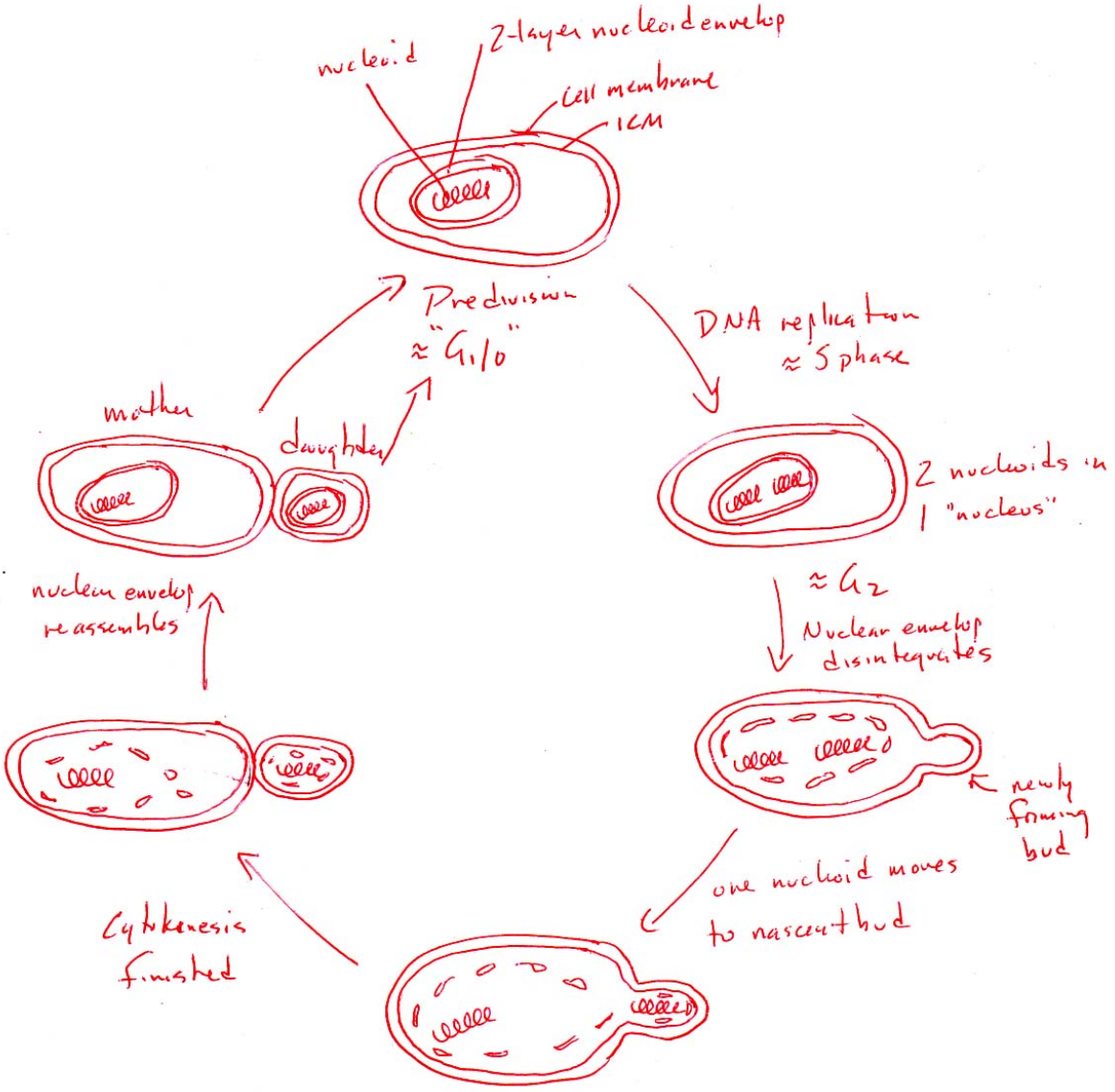
But when they used PCR and 16S rRNA analysis with universal primers, the only sequence they got was that of Igniococcus. A fluorescent probe against this sequence lights up only the Igniococcus cells, not the small cocci. However, in Southern blotts, they could see that these small cocci did contain 16S rRNA genes - they just wouldn't amplify with the usual primers. So they cloned and sequenced the 16S rRNA directly from the band on the Southern gel. The sequence is very unusual, thus the failure of the universal primers to amplify it. They confirmed their data by making a FISH probe based on this sequence, that lights up only the small cocci, not the host. Trees of the sequence show it to be clearly archaeal, but apparently not belonging to either the Crenarchaea nor the Euryarchaea; it is a 4th phylogenetic kind of archaeon. They characterized the organism and named it Nanoarchaeum equitans.

Conclusion : N. equitans can only grow stuck to Igniococcus; no other host ill do. They say that it doesn't seem to slow the growth of it's host, but in a later paper they say it does slow the growth of it's host when present in larger numbers. So it's a parasite. This is the first hyperthermophilic symbiont known, and also the first archaeal parasite. It is also the smallest cellular organism known, and has the smallest genome known, just shy of 500,000bp.

(Many other answers are possible)

23. Planctomycetes divide by budding, and one planctomycete, *Gemmata*, has a membrane enclosed nucleoid. Given what you know about chromosome structure and replication in Bacteria (from *Caulobacter*), diagram/describe how you imagine chromosome replication and cell division might work in *Gemmata*. How would you test your ideas experimentally?

One way this might work could be according to the stereotypical eukaryotic format - like, say, yeast. The DNA could be replicated, then the "nucleoid" envelop dispersed. The sister chromosomes could then be partitioned, one of them to the end of the cell where asymmetric cytokinesis (fission) is just beginning to form a small bud. Whatever pulls the chromosomes around would pull that chromosome into the bud, which could then finish pinching off to complete bud formation. Once cell division is complete, the nucleoid envelops could reorganize around the nucleoids of both the mother and daughter cells.



The straightforward, although perhaps tedious, way to look at this would be via electron microscopy. It might help to start by fractionating cells in a culture by size - the smallest cells would be new buds. A new culture could be started with these fresh buds, which would grow synchronously. At various times, samples could be removed for microscopy (so you know what they're doing) and EM. As cells progress through the division cycle, you could see what the nucleoid envelop 'behaves', see how the nucleoid partitions, how cyctokinesis works. To look at DNA replication, you might have to use DAPI staining (the amount of dye bound should double during replication) or look for the incorporation of radioactively-labeled precursors into DNA.

(Many other answers are possible)

List or genera extracted from the lecture notes

Acetobacterium
Acidianus
Agmenellum
Agrobacterium
Alcaligenes
Alteromonas
Anabaena
Anacystis
Angiococcus
Aphanizomenon
Aquifex
Aquifex
Archaeoglobus
Arthrobacter
Arthromitis
Arthrospira
Azoarcus
Azotobacter
Bacillus
Bdellovibrio
Blaberus
Blatta
Bradyrhizobium
Brocadia
Brucella
Burkholderia
Caldotoga
Campylobaacter
Caulobacter
Chalymdophila
Chlamydia
Chromatium
Clostridium
Comamonas
Copia
Corynebacterium
Criblamydia
Deinococcus
Desulfovibrio
Desulfurococcus
Desulfurolobus
Desulfuromonas
Enterococcus
Escherichia
Eubacterium
F
Fervidobacterium
Frankia
Gemmata
Geotoga
Haemophilus
Halobacterium
Haloferax
Hartmanella
Helicobacter
Heliobacterium
Hyphomicrobium
Igniococcus
Isosphaera
Kalotermes
Kuenenia
Lactobacillus
Legionella
Leuconostoc
Listeria
Lyngbya
M13
Marinotoga
Megasphaera
Metallosphaera
Methanobacterium
Methanococcus
Methanomicrobium
Methanopyrus
Methanosarcina
Methanospirillum
Methanothermobacter
Micrococcus
Microcoleus
Mimivirus
Mu
Mycobacterium
Mycoplasma
Myxococcus
Nanoarchaeum
Neochlamydia
Niesseria
Nitrobacter
Nitrosococcus
Nitrosomonas
Nocardia
Nodularia
Nostoc
P element
Parachlamydia
Peptostreptococcus
Petrotoga
Pirellula
Planctomyces
Porcellio
Prochlorococcus
Prochloron
Proteus
Protochlamydia
Pseudomonas
Psosthecobacter
Pterotermes
Pyrobaculum
Pyrococcus
Pyrodictium
Reticulitermes
Rhabdochlamydia
Rhizobium
Rhodobacter
Rhodomicrobium
Rhodopseudomonas
Rhodospirillum
Rickettsia
Rivularia
Rubrivivax
Sarcina
Serratia
Shewanella
Simkania
Spirillum
Sporomusa
Staphylococcus
Staphylothermus
Stigmatella
Streptococcus
Streptomyces
Stygiolobus
Sulfolobus
T4
Thauera
Thermococcus
Thermocrinus
Thermodiscus
Thermofilum
Thermopallium
Thermoplasma
Thermoproteus
Thermosipho
Thermotoga
Thiobacillus
Ty
Ultramicrobium
UWE25
Verrucomicrobium
Vibrio
Vitrioscilla
Waddlia

Papers discussed in class:

Horn M, et al., 2004 Illuminating the evolutionary history of *Chlamydiae*. Science 304:728-730

Nelson KE, et al., 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. Nature 399:323-329.

Venter JC, et al., 2004 Environmental genome shotgun sequencing of the Sargasso sea. Science 304:66-74.

Margulis L, Jorgensen JZ, Dolan S, Kolchinsky R, Rainey FA, & Lo S-C. 1998 The *Arthromitis* stage of *Bacillus cereus*: Intestinal symbionts of animals. Proc. Natl. Acad. Sci. USA 95:1236-1241.

Huber H, Hohn MJ, Rachel R, Fuchs T Wimmer VC and Stetter KO. 2002 A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417:63-67

Xie & Reeve 22004 Transcription by an archaeal RNA polymerase is slowed but not blocked by an archaeal nucleosome. J. Bacteriol. 186:3492-8

Strous, et al., 2006 Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440:790-4

Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, McAdams HH & Shapiro L. 2004 Rapid and sequential movement of individual chromosomal loci to specific subcellular locations. Proc. Natl. Acad. Sci. USA 101:9257-9262

Miguélez EM, Hardissson C, and Manzanal MB. 1999 Hyphal death during colony development in *Streptomyces antibioticus*: Morphological evidence for the existence of a process of cell deletion in a multicellular prokaryote. J. Cell Biol. 145:515-525.

Dale Kaiser & Roy Welch 2004 Dynamics of fruiting body morphogenesis. J. Bacteriol. 186:919-27

Soto C and Castilla J 2004 The controversial protein-only hypothesis of prion propagation. Nature Medicine 10:S63-S67

Somerville RA 2002 TSE agent strains and PrP: Reconciling structure and function. TiBS 27:606-612