Teacher Resource for:

Molecular Basis for the Nerve Dependence of Limb Regeneration in an Adult Vertebrate

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GENERAL USE OF *Science* in the Classroom

Student Learning Goals:

"One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge."[1]

The U.S. National Academy of Sciences defines science as: "Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim."

An important student learning goal, central to any understanding of "the nature of scientific knowledge," is to give each student an appreciation of how science is done.

This includes knowing why:

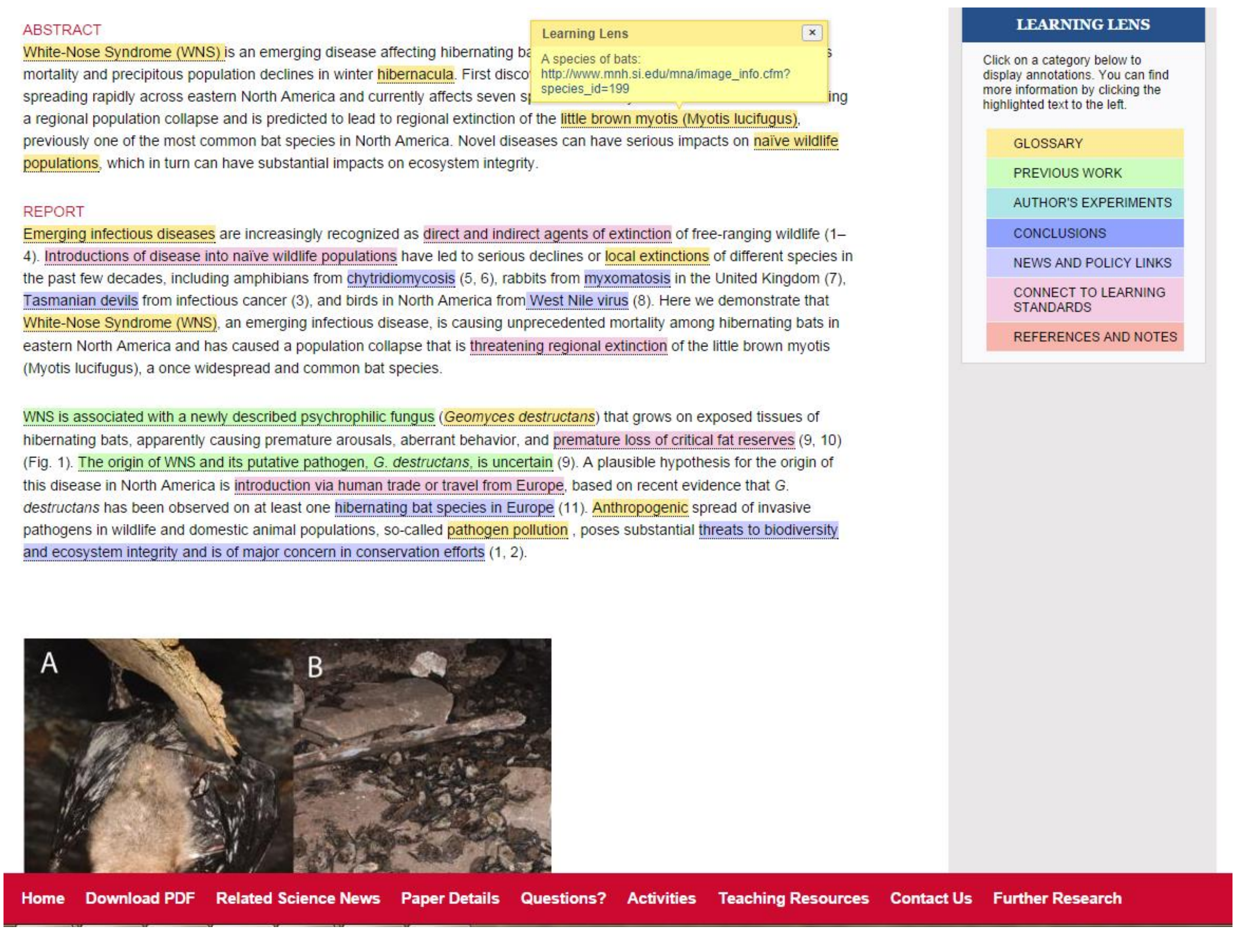
- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist's conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

[1] *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

Learning Lens:

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.



An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

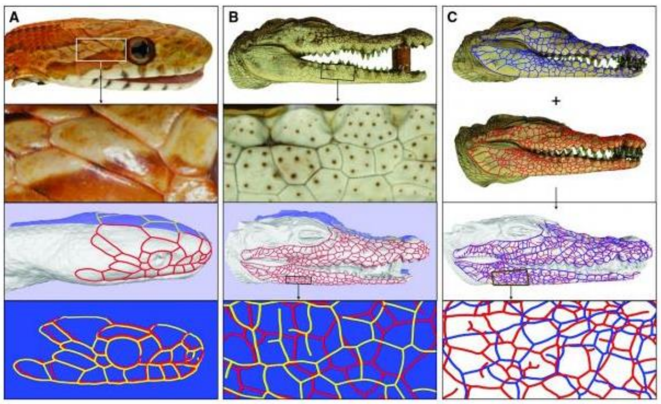


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). (B) Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. (C) Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).

Method: 3D geometry and color-texture reconstruction | Panel A | Panel B | Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

- GLOSSARY
- PREVIOUS WORK
- AUTHOR'S EXPERIMENTS
- CONCLUSIONS
- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

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References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

6. L. Lewejohann, B. Zipser, N. Sachser, "Personality" in laboratory mice used for biomedical research: A way of understanding variability? Dev. Psychobiol. 53, 624 (2011).
7. G. Kempermann, The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163 (2008).
8. Lewejohann et al., Behavioral phenotyping of a murine model of Alzheimer's disease in a seminaturalistic environment using RFID tracking. Behav. Res. Methods 41, 850 (2009).
9. B. Steineret al., Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41 (2004).
10. Garthe, J. Behr, G. Kempermann, Ad … flexible use of spatially precise learning strategies. PLoS ONE 4, e54…
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12. G. Kempermannet al., Why and how … brain plasticity. Front Neurosci 4, 189 (2010).
13. K. Fabelet al., Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front. Neurosci. 3, 50 (2009).
14. Amrein, H. P. Lipp, Adult hippocampal neurogenesis of mammals: Evolution and life history. Biol. Lett. 5, 141 (2009).
15. G. Kempermann, Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci. 22, 635 (2002).
16. C. Crabbe, D. Wahlsten, B. C. Dudek, Genetics of mouse behavior: Interactions with laboratory environment. Science 284, 1670 (1999).
17. Lewejohann et al., Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav. 5, 64 (2006).
18. D. W. Bailey, How pure are inbred strains of mice? Immunol. Today 3, 210 (1982).
19. R. Lathe, The individuality of mice. Genes Brain Behav. 3, 317 (2004).
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21. R. P. Talenset al., Epigenetic variation during the adult lifespan: Cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694 (2012).
22. P. B. Baltes, J. R. Nesselroade, S. W. Cornelius, Multivariate antecedents of structural change in development: A simulation of cumulative environmental patterns. Multivariate Behav. Res. 13, 127 (1978).
23. M.E. Raijmakers, P. C. Molenaar, Modeling developmental transitions in adaptive resonance theory. Dev. Sci. 7, 149 (2004).
24. K. Friston, M. Breakspear, G. Deco, Perception and self-organized instability. Front. Comput. Neurosci. 6, 44 (2012).
25. Van de Weerd et al., Effects of environmental enrichment for mice: Variation in experimental results. J. Appl. Anim. Welf. Sci. 5, 87 (2002).
26. D. P. Wolferet al., Laboratory animal welfare: Cage enrichment and mouse behaviour. Nature 432, 821 (2004).
27. K. Lewin, Dynamic Theory of Personality (McGraw-Hill, New York, 1935).
28. K. L. Jang, R. R. McCrae, A. Angleitner, R. Riemann, W. J. Livesley, Heritability of facet-level traits in a cross-cultural twin

Learning Lens ×

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

LEARNING LENS

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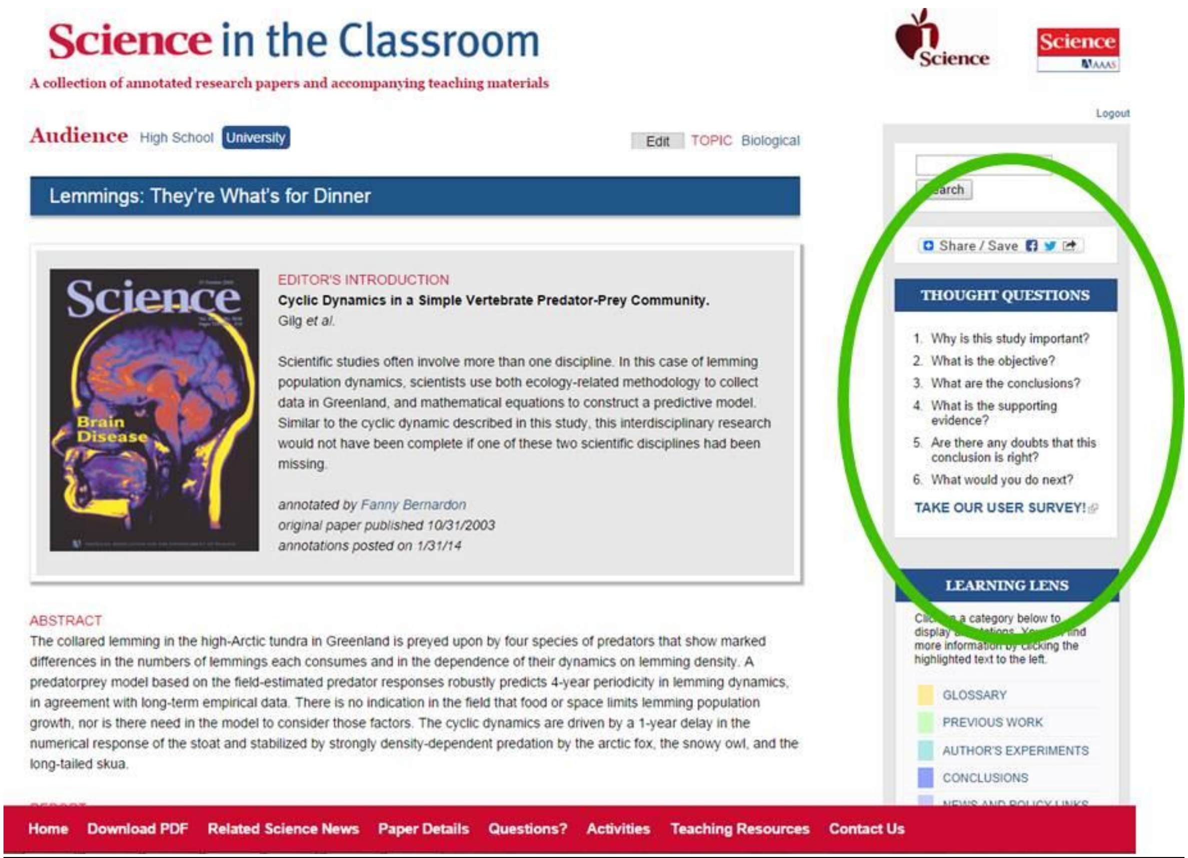
- REFERENCES AND NOTES

Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.



Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.

2. Activities tied to the articles.

Some ways to use the *Science* in the Classroom articles:

1. Assign to student groups to read and discuss during class.

2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.

3. Assign to individual students to complete during class or as homework.

4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).

2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.

3. Have students edit the article, or parts of the article, to a simpler reading level.

4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier

publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.

6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- How are animals able to regenerate structures?
- How do molecules drive regeneration, growth, and differentiation?
- How are some animals able to regenerate structures, but others are not?

The importance of this scientific research

- Understanding of how limb regeneration is dependent on nerves at the molecular level and how those molecules influence limb regeneration.

The actual science involved

- Regeneration biology
- Molecular biology
- Cell biology

Connect to Learning Standards:

The Next Generation Science Standards

- Practice 2: Developing and using models
- Practice 6: Constructing explanations (for science) and designing solutions (for engineering)
- Practice 7: Engaging in argument from evidence
- Practice 8: Obtaining, evaluating, and communicating information

The AP Biology Standards

- Essential knowledge 1.B.2: Phylogenetic trees and cladograms are graphical representations (models) of evolutionary history that can be tested.
- Essential knowledge 2.D.3: Biological systems are affected by disruptions to their dynamic homeostasis.
- Enduring understanding 2.E: Many biological processes involved in growth, reproductionb and dynamic homeostasis include temporal regulation and coordination.
- Essential knowledge 3.B.1: Gene regulation results in differential gene expression, leading to cell specialization.
- Essential knowledge 3.D.2: Cells communicate with each other through direct contact with other cells or from a distance via chemical signaling.
- Practice 1: The student can use representations and models to communicate scientific phenomena and solve scientific problems.
- Practice 6: The student can work with scientific explanations and theories.

Common Core English Language Arts

- 11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11–12 texts and topics.
- 11-12.6: Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

Salamanders are known for having amazing regenerative ability, especially when compared with other vertebrates such as humans. Many studies have been done in the early 20th century that attempt to explain exactly how these animals are able to regenerate in regard to their limbs. One important finding was that these animals could not regenerate their limbs if the nerve of the limb is denervated (destroyed, removed, or cut). Because these studies were done before our current understanding of the molecular basis of biology, it was not known why lack of a nerve prevents a salamander from regenerating its limb. This paper addresses this old finding by attempting to identify an exact molecule that explains this observation. The authors find that the presence of protein nAG is dependent on an intact nerve. More importantly, they demonstrate that exogenously adding back nAG to a limb with a severed nerve allows for proper limb regeneration, rescuing the phenotype. These data taken together suggest that nAG may be the molecular mechanism underlying nerve dependent limb regeneration.

Topics Covered:

- Protein secretion and membrane proteins
- Cell signaling
- Imaging and staining techniques
- Molecular cloning
- Bacterial and vertebrate cell culture

Why this research is important:

Understanding regeneration is of crucial importance to human health. With the presence of disease, war, and birth defects, many people have lost limbs or have ones that are nonfunctional. This of course does not take into account the numerous people that have other degraded/missing structures such as in neurodegenerative disease. In turn, being able to regenerate proper, working limbs and structures would be of critical importance to human health and quality of life. Results from this paper describe a molecular mechanism underlying regeneration and will allow us to translate (apply) this information to organisms more closely related to humans such as mice and primates. Information from these studies will hopefully one day provide insights into how humans can regenerate their limbs.

Methods used in the Research:

- Immunoblotting (westerns)
- Yeast two-hybrid screens
- Phylogenetic analysis
- Microscopic imaging
- Immunohistochemistry
- Animal methods (i.e., surgery)
- Cell culture

Conclusions:

- nAG is a secreted protein that binds to the receptor protein Prod1.
- Homologous proteins to nAG are found in a variety of vertebrate species.
- nAG is expressed in low levels in the intact limb, but on amputation, expression levels increase.
- In animals with denervated limbs, nAG expression is not present.
- Ectopic expression of nAG in the denervated limb rescues limb regeneration. However, the nerve and muscle tissues do not regenerate properly.
- nAG, in vitro, appears to increase the number of cells going through S-phase.

Areas of Further Study:

- How can this research be applied to humans?
- What is the function of nAG homologs in other vertebrates?
- What is the role of the other identified Prod1 ligand in regeneration?

Discussion Questions:

Discussion questions associated with the learning standards

Practice 1: Asking questions (for science) and defining problems (for engineering)
Practice 3: Planning and carrying out investigations

- Another molecule that might bind to Prod1 was identified in the authors' yeast two-hybrid screen. This protein, although not named, was mentioned as being part of the resistin family of proteins. If you were a scientist, what are some experiments you would perform to test this other protein?

Practice 2: Developing and using models

- How would these experiments have differed if they were done in a mouse? Or better yet, would it even be possible to do these experiments? What are the pros and cons of using either model organism?

Vision and Change Core Competencies and Disciplinary Practice: Competency 6: Ability to understand the relationship between science and society

- The authors identify nAG as a potential molecule driving limb regeneration. What would be the societal impact if nAG or a similar protein was found to be able to regenerate human limbs? Explain using your knowledge of biotechnology how a nAG based therapy could be made.

Essential knowledge 1.A.2: Natural selection acts on phenotypic variations in populations.

- What benefit does regeneration provide for salamanders? Why, evolutionarily, would it not make sense for humans to have regenerative ability? Propose an explanation of how, at the molecular level, humans have lost their regenerative ability. Humans have proteins similar to nAG; how can the proteins be similar but the functions not?

Additional Material:

Figures and data

- Figure 1: A cross section of the limb blastema of an adult newt. Look at the scale bar (bottom right), you will see the limb was about 2 mm in diameter. Note the densely packed cells of the cone-shaped blastema. The plane of transection was about 500 microns proximal to the tip of the cone. The nerve is the pink-stained tongue of tissue on the right-hand side, marked N. The blastema is at about 15 days posttransection of the limb.

- Figure 2: Light microscope image of glands (one marked G) under the epidermis of a normal limb. Note the change in scale relative to Figure 1.

- Figure 3: A scanning electron micrograph of a regenerating newt limb. The original transection plane is arrowed. Note that the limb stump epidermis (above the plane) has periodic ducts opening onto the surface. These discharge the contents of underlying glands. The wound epidermis (below the plane) does not have such ducts. The nAG-containing glands considered in the paper are ductless, and discharge their contents internally. Estimate the diameter of a duct.

- Figure 4: Transmission electron micrograph of a secretory granule in a gland cell underlying the wound epidermis. The section was reacted with antibodies to nAG, and then with a secondary antibody labeled with gold particles (black dot). The scale bar is 0.5 microns, and the gold particles are 10 nanometers. Note that gold particles are also localized on the endoplasmic reticulum as well as the granule. Why is this ?

Points for discussion

1. The problem of nerve dependence presents a fascinating paradox that is only hinted at in the paper. A limb that develops without a nerve supply, called an ANEUROGENIC LIMB (AL), can regenerate without innervation! Most scientists are quite surprised when confronted with this finding. If nerves are allowed to grow into an AL, then nerve dependence is acquired. So nerve dependence is "imposed" by the nerve during normal limb development.
Can you come up with a model that could account for these findings, based on what you have learned from the paper?

nAG is expressed in the developing limb epidermis at high levels. The nerve grows in and permanently down-regulates nAG. Therefore regeneration requires a new source based on nerve regeneration as outlined in the paper. The AL has a strong source of nAG, in its epidermis, for regeneration, and does not need the nerve.
Ref Kumar et. al. Proc Nat Acad Sci 108, 13588-93 (2011)
"The aneurogenic limb identifies developmental cell interactions underlying vertebrate limb regeneration."

2. Nerve dependence of regeneration of complex structures has turned out to be widespread in invertebrates and vertebrates. Why do you think this is?

Regeneration of such structures demands a big investment of materials and energy. This is wasted unless the regenerate is innervated. Nerve dependence ensures that the nerve must be regenerating at the same time as formation and growth of the blastema.