BIOGRAPHICAL SKETCH

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NAME: Johnson, Carl Hirschie

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POSITION TITLE: Cornelius Vanderbilt Professor of Biological Sciences, Vanderbilt University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Texas at Austin	B.A.	06/1976	Honors Liberal Arts ("Plan II")
Stanford University	Ph.D.	01/1982	Biology
Harvard University	Postdoc	07/1987	Cell & Develop. Biology

A. Personal Statement

The goal of this project is to investigate the mechanism and evolution of biological timing systems, including circadian clocks and photoperiodic time measurement. I propose to take advantage of the unique capabilities of model systems to address these important questions, namely cyanobacteria and mice. I have the experience, expertise, leadership, energy, and motivation necessary to successfully carry out the proposed work. I have studied circadian rhythms since my undergraduate work in a variety of organisms and using techniques ranging from structural biology, biochemistry, and cell biology, to electrophysiology, genetics, behavior, and population biology. I have been trained by leaders in the field: my postdoctoral supervisor was J.W. Hastings, a leader of molecular studies of circadian clocks, my graduate supervisor was Colin Pittendrigh, a "founder" of the field, and my undergraduate supervisor (who inspired me to seek a Ph.D. rather than an M.D.) was Michael Menaker, a leader in the study of circadian behavior and physiology in vertebrates. While the use of mice as a model system requires no explanation, a justification for cyanobacteria as a model system is perhaps appropriate. Cyanobacteria are particular amenable to the study of circadian clock mechanisms and evolution because of unique genetic and molecular capabilities. Within a relatively short period of time, this model system for studying clocks has progressed from obscurity/disbelief (25 years ago, chronobiologists were convinced that prokaryotes were not capable of circadian phenomena!) to becoming one of the preeminent systems for studying circadian rhythmicity (arguably, the best understood in terms of its biochemistry, biophysics, and evolutionary fitness). My laboratory is one of the key labs that orchestrated the transformation of cyanobacteria into a clock model; our initial collaboration with the laboratories of Drs. Takao Kondo and Susan Golden led to the establishment of the *Synechococcus elongatus* system (we still maintain a friendly and supportive competition & collaboration with the Kondo and Golden groups). There have been a number of significant innovations and surprising insights that have come from the study of the cyanobacterial clock system, several of which are described in Section C of this BioSketch.

At Vanderbilt University, I have been instrumental in building a vibrant group of circadian/sleep researchers at Vanderbilt that interact regularly, especially the labs of myself, Dr. Douglas McMahon (circadian rhythms in mammalian SCN and retina), and Dr. Beth Malow (human sleep). Finally, because of my long association with the circadian field and the fact that we study organisms other than cyanobacteria, my laboratory takes a “bigger picture” in the analysis of the cyanobacterial clock than might be true of a laboratory that specializes on cyanobacteria. For example, my laboratory has also published on rhythmic phenomena in mammalian cell cultures, mouse behavior, human genetics & metabolism, microbiomes, high-throughput screening, yeast metabolism, and plant signal transduction. This broad view means that our study of cyanobacteria is always

tempered by the attempt to elucidate themes that are potentially applicable to other systems and are not merely details that are specific to cyanobacteria. The following are publications that highlight the breadth of my experience and qualifications for this project (as a separate perspective from the publications presented in Section C of this BioSketch):

Zhao C, Kelly K, Jabbur ML, Paguaga M, Behringer M, **Johnson CH.** 2022. Host circadian behaviors exert only weak selective pressure on the gut microbiome under stable conditions but are critical for recovery from antibiotic treatment. *PLoS Biology* 20: e3001865. PMCID: PMC 9645659

Kelly KP, Borsetti H, Wenzler ME, Ustione A, Kim K, Christov PP, Ramirez B, Bauer JA, Piston DW, **Johnson CH**,** Sulikowski GA**. 2022. Screen for Small-Molecule Modulators of Circadian Rhythms Reveals Phenazine as Redox-State Modifying Clockwork Tuner. *ACS Chemical Biology* 17: 1658-1664. **co-corresponding authors. PMCID: PMC9398883

Kelly KP, Ellacott KJ, Chen H, McGuinness OP, and **Johnson CH**. 2021. Time-Optimized Feeding is Beneficial Without Enforced Fasting. *Open Biology* 11: 210183. PMCID: PMC8492177

McNaughton CD, Adams NM, **Johnson CH**, Ward MJ, Schmitz JE, Lasko TA. 2021. Diurnal variation in SARS-CoV-2 PCR test results: Test accuracy may vary by time of day. *J. Biological Rhythms* 36: 595-601. PMCID: PMC8599649

Kelly, K.P., O.P. McGuinness, M. Buchowski, J.J. Hughey, H. Chen, J. Powers, T. Page, **C.H. Johnson**. 2020. Eating breakfast and avoiding late-evening snacking sustains lipid oxidation in humans. *PLoS Biology* 18: e3000622. PMCID: PMC7046182

Shi, S.-Q, M.J. White**, H.M. Borsetti**, J.S. Pendergast, **A. Hida**, C.M. Ciarleglio, P.A. de Verteuil, A.G. Cadar, **C. Cala**, D.G. McMahon, R.C. Shelton, S.M. Williams, **C.H. Johnson**. 2016. Molecular Analyses of Human Circadian Gene Variants Reveal Sex-dependent Links Between Depression and Clocks. *Translational Psychiatry* 6: e748. PMCID: PMC4872462

Shi, S., T.J. Bichell, R.A. Ihrie, **C.H. Johnson**. 2015. Ube3a Imprinting Impairs Circadian Robustness in Angelman Syndrome Models. *Current Biology* 25: 537–545. PMCID: PMC4348236

Ciarleglio, C.M., K. Ryckman, **S.V. Servick, A. Hida,** S. Robbins, N. Wells, J. Hicks, S.A. Larson, J.P. Wiedermann, K. Carver, N. Hamilton, K.K. Kidd, J.R. Kidd, J.R. Smith, J. Friedlaender, D.G. McMahon, S. Williams, M.L. Summar, and **C.H. Johnson**. 2008. Genetic Differences in Human Circadian Clock Genes Among Worldwide Populations. *J. Biol. Rhythms* 23: 330-340. PMCID: PMC2579796

Johnson, C. H., M. R. Knight, T. Kondo, P. Masson, J. Sedbrook, A. Haley, and A. Trewavas. 1995. Circadian oscillations of cytosolic and chloroplastidic free calcium in plants. *Science 269*: 1863-1865.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1999-present	Professor, Department of Biological Sciences, Vanderbilt University
1994-99	Associate Professor, Department of Biology, Vanderbilt University
1987-94	Assistant Professor, Department of Biology, Vanderbilt University

Awards and Other Professional Activities

2019-	Cornelius B. Vanderbilt Professor of Biological Sciences (endowed professorship)
2015-	MERIT Award from the National Institute of General Medical Sciences (R37 GM067152)
2014	Aschoff and Honma Prize in Biological Rhythm Research
2012-2014	President, Society for Research on Biological Rhythms (SRBR)
2011-2018	Stevenson Professor of Biological Sciences (endowed professorship)
2007-present	Professor (Secondary Faculty), Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center
2005	Chancellor's Research Award, Vanderbilt University
1996-2000	Secretary & Treasurer, Society for Research on Biological Rhythms
1995-2020	Editorial Advisory Board, *Journal of Biological Rhythms*
1994-2004	Research Scientist Development Award from the NIMH (K02 MH01179)
1992	Ampere Fellowship, Ibaraki University, Japan
1988	Visiting Lecturer, University of Tsukuba, Japan

1986 Jean and Katsuma Dan Fellow (for 3 months research in Japan)
1983 MBL Analytical and Quantitative Light Microscopy Course, MBL, Woods Hole, MA
1982-85 National Institutes of Health Postdoctoral Fellowship (NRSA F32 GM08288)
1979 Marine Biological Laboratory Physiology Course (Woods Hole, MA)
1976-79 National Science Foundation Graduate Fellowship
1976 Ettlinger Award for Outstanding Plan II student (University of Texas)
1975 Phi Beta Kappa, University of Texas at Austin

C. Contributions to science (other than the current project of this proposal)

1. Discovery/development of the circadian system in cyanobacteria. As mentioned above, prior to our work, chronobiologists were convinced that prokaryotes were not capable of circadian phenomena. We developed a luciferase reporter strain of cyanobacteria and used it to demonstrate unequivocally that these prokaryotes exhibit all the canonical clock properties. The reporter strain was then used in a high-throughput mutagenesis screen to identify the central clock genes, *kaiA, kaiB*, and *kaiC*. These studies laid the groundwork for the discovery of a coupled oscillator system in cyanobacteria composed of a biochemical post-translational oscillator (which can be reconstituted *in vitro*) and a transcription/translation feedback loop. This system is robust to metabolic and environment conditions and has established new criteria for circadian clock mechanisms that have considerably influenced conclusions about the circadian mechanisms in eukaryotes. Therefore, from initial disbelief, this prokaryotic circadian system is now one of the preeminent systems for studying circadian rhythmicity, especially at the molecular level.

Kondo, T., C. A. Strayer, R. D. Kulkarni, W. Taylor, M. Ishiura, S. S. Golden, and **C. H. Johnson**. 1993. Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. Proc. Natl. Acad. Sci. USA *90*: 5672-5676. PMCID: PMC46783

Ishiura, M., S. Kutsuna, S. Aoki, H. Iwasaki, C. R. Andersson, A. Tanabe, S. S. Golden, **C. H. Johnson**, and T. Kondo. 1998. Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria. Science *281*: 1519-1523. PMID: 9727980

Qin, X., M. Byrne, Y. Xu, T. Mori, and **C.H. Johnson**. 2010. Coupling of a core post-translational pacemaker to a slave transcription/translation feedback loop in a circadian system. *PLoS Biology* 8: e1000394. PMID: 20563306; PMCID: PMC2885980.

Johnson, C.H., C. Zhao, Y. Xu, and T. Mori. 2017. Timing the day: what makes bacterial clocks tick? *Nature Reviews Microbiology* 15: 232-242. PMCID: PMC5696799

Jabbur ML, **Johnson CH.** 2024. Bacteria can anticipate the seasons: photoperiodism in cyanobacteria. *Science* 385: 1105–1111. PMID: 39236161

2. Adaptive significance: Chronobiologists believe that circadian systems confer an adaptive advantage, but my lab was the first to rigorously test the adaptive fitness of circadian rhythmicity (using the cyanobacterial system). We used an innovative competition assay to show that strains of cyanobacteria whose clock properties (e.g., period) optimally matched environmental conditions could out-compete strains whose clock properties did not match the environment. Delightfully, even mutant strains can out-compete wild-type strains if the mutant's properties were matched more appropriately to the environment.

Ouyang, Y., C.R. Andersson, T. Kondo, S.S. Golden, and **C.H. Johnson**. 1998. Resonating circadian clocks enhance fitness in cyanobacteria. *Proc. Natl. Acad. Sci. USA* 95: 8660-8664. PMCID: PMC21132

Woelfle, M.A., Y. Ouyang, K. Phanvijhitsiri, and **C.H. Johnson**. 2004. The adaptive value of circadian clocks: An experimental assessment in cyanobacteria. *Current Biology* 14: 1481–1486. PMID: 15324665

Edgar, R.S., E.W. Green, **Y. Zhao**, G. van Ooijen, M. Olmedo, **X. Qin, Y. Xu**, M. Pan, U.K. Valekunja, K.A. Feeney, E.S. Maywood, M.H. Hastings, N.S. Baliga, M. Merrow, A.J. Millar, **C.H. Johnson**, C.P. Kyriacou, J.S. O'Neill, A.B. Reddy. 2012. Peroxiredoxins are conserved markers of circadian rhythms. *Nature* 485: 459-64. PMCID: PMC3398137

Ma, P., T. Mori, C. Zhao, T. Thiel, **C.H. Johnson**. 2016. Evolution of KaiC-dependent timekeepers: a proto-circadian timing mechanism confers adaptive fitness in the purple bacterium *Rhodopseudomonas palustris. PLoS Genetics* 12: e1005922. PMCID: PMC4794148

Jabbur ML, Dani C, Spoelstra K, Dodd AN, **Johnson CH.** 2024. Evaluating the Adaptive Fitness of Circadian Clocks and their Evolution. *Journal of Biological Rhythms* 39: 115–134. PMCID: PMC10994774

3. The first concerted application of structural biology techniques to the study of circadian proteins. We were the first to study the structural properties of clock proteins, e.g., using analytical ultracentrifugation, electron microscopy (EM), and X-ray crystalization of the central clock protein KaiC. We used these approaches to elucidate circadian mechanisms, e.g., by EM of clock protein complexes to clarify the mechanism by which the *in vitro* circadian oscillator "ticks," and by identifying the phosphorylation reactions of KaiC. This information was used to derive the first comprehensive mathematical models that can accurately simulate the *in vitro rhythms* and provide concrete predictions for experimental testing.

Pattanayek, R., J. Wang, **T. Mori, Y. Xu, C.H. Johnson**, and M. Egli. 2004. Visualizing a circadian clock protein: crystal structure of KaiC and functional insights. *Molecular Cell* 15: 375–388. PMID: 15304218

Xu, Y., T. Mori, R. Pattanayek, S. Pattanayek, M. Egli, and **C.H. Johnson**. 2004. Identification of Key Phosphorylation Sites in the Circadian Clock Protein KaiC by Crystallographic and Mutagenetic Analyses. *Proc. Natl. Acad. Sci. USA* 101: 13933-13938. PMCID: PMC518856

Johnson, C.H., M. Egli, P.L. Stewart. 2008. Structural Insights into a Circadian Oscillator. *Science* 322: 697-701. PMCID: PMC2588432

Mori, T., S. Sugiyama, **M. Byrne**, **C.H. Johnson**, T. Uchihashi, T. Ando. 2018. Revealing circadian mechanisms of integration and resilience by visualizing clock proteins working in real time. *Nature Commun.* 9: 3245. PMCID: PMC6092398

4. BRET (Bioluminescence Resonance Energy Transfer): We invented and developed the BRET methodology as a direct result of our interest in circadian biology. In particular, we considered using a FRET approach to study the interaction of circadian proteins, but we wanted to avoid fluorescence illumination that could reset the phase of the circadian clock (light will cause phase resetting of most circadian clocks). From a solid grounding in bioluminescence, I realized that in nature, the function of Green Fluorescent Protein (GFP) is to serve as a BRET partner to a luciferase (aequorin). This led to the realization that perhaps BRET could replace FRET in applications where fluorescence excitation was undesirable. That insight led to our invention of BRET, which was granted a patent from the European Patent Office, and we are currently expanding the development of BRET sensors for Ca^{++} and neural activity. We are adapting our new BRET reporter for Ca^{++} fluxes for innovations in high-throughput screening applications.

Xu, Y., D. Piston, and **C.H. Johnson**. 1999. A bioluminescence resonance energy transfer (BRET) system: Application to interacting circadian clock proteins. *Proc. Natl. Acad. Sci. USA 96*: 151-156. PMCID: PMC15108

Yang, J., D. Cumberbatch, S. Centanni, **S. Shi**, D. Winder, D. Webb, **C.H. Johnson.** 2016. Coupling Optogenetic Stimulation with NanoLuc-based Luminescence (BRET) Ca^{++} Sensing. *Nature Communications* 7: 13268. PMCID: PMC5476805

Yang J, and **Johnson CH.** 2021. Bioluminescent sensors for Ca^{++} flux imaging and the introduction of a new intensity-based Ca^{++} sensor. *Frontiers in Bioengineering and Biotechnology* 9: 773353. PMCID: PMC8578923

Cumberbatch D, Mori T, Yang J, Mi D, Vinson P, Weaver D, **Johnson CH**. 2022. A BRET Ca^{2+} sensor enables high-throughput screening in the presence of background fluorescence. *Science Signaling* 15: eabq7618. PMCID: PMC9930640

5. Global circadian gene expression: We discovered that the circadian system in cyanobacteria regulates the expression of EVERY promoter in the organism as an output of the clock. This global expression is mediated in part by oscillating topology of the entire chromosome. These investigations also led us to the unexpected discovery of codon usage bias as a mechanism for circadian conditionality. We have taken advantage of these discoveries to enlist circadian clock properties to maximize cost-effective expression of useful bioproducts such as biopharmaceuticals and biofuels in cyanobacteria, and we have a patent application that has just been allowed that introduces that concept (U.S. Pat. Appl. No. 14/933,622).

Liu, Y, N.F. Tsinoremas, **C.H. Johnson**, N.V. Lebedeva, S.S. Golden, M. Ishiura, and T. Kondo. 1995. Circadian orchestration of gene expression in cyanobacteria. *Genes and Development* 9: 1469-1478. PMID: 7601351

Xu, Y., P.D. Weyman, M. Umetani, **J. Xiong, X. Qin**, Q. Xu, H. Iwasaki, and **C. H. Johnson**. 2013. Circadian Yin-Yang regulation and its manipulation to globally reprogram gene expression. *Current Biology* 23: 2365-2374. PMCID: PMC3889637

Xu, Y., P. Ma, P. Shah, A. Rokas, Y. Liu, **C.H. Johnson**. 2013. Non-optimal codon usage is a mechanism to achieve circadian clock conditionality. *Nature* 495: 116-20. PMCID: PMC3593822

Xu Y, Jabbur ML, Mori T, Young JD, **Johnson CH**. 2024. Clocking Out and Letting Go to Unleash Green Biotech Applications in a Photosynthetic Host. *Proc. Natl. Acad. Sci. USA* 121: e2318690121. PMCID: PMC11127020

Books:

2009 Bacterial Circadian Programs, J.L. Ditty, S.R. Mackey, C.H. Johnson, eds. (Springer), 333 pages.

2021 Circadian Rhythms in Bacteria and Microbiomes, C.H. Johnson and M. Rust, eds. (Springer Nature), 415 pages.

Complete List of Published Work in MyBibliography (currently 192 total):

http://www.ncbi.nlm.nih.gov/sites/myncbi/carl.johnson.1/bibliography/40700187/public/?sort=date&direction=ascending