## BIOGRAPHICAL SKETCH

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NAME: BREWER, Gary

eRA COMMONS USER NAME (credential, e.g., agency login): BREWERGA

POSITION TITLE: Professor of Biochemistry & Molecular Biology

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 |
|---|---|---|---|
| Auburn University, Auburn, AL | B.S. | 06/1976 | Physics |
| Vanderbilt University School of Medicine, Nashville, TN | Ph.D. | 08/1984 | Biochemistry |
| McArdle Laboratory for Cancer Research, Univ. of Wisconsin, Madison, WI | Postdoctoral | 08/1989 | Molecular Biology |

### A. Personal Statement

In 1989 we began a research program to understand how cells regulate mRNA levels for proto-oncogenes encoding transcription factors are regulated. This was an important question since abnormal expression of these genes leads to neoplastic phenotypes. A characteristic of most proto-oncogene mRNAs is that present extremely short half-lives. For over 20 years, we have been interested in the mechanisms responsible for rapid degradation of proto-oncogene mRNAs and identifying the factors involved in their degradation. These questions led to our 1993 discovery of the RNA-binding protein AUF1, which controls degradation and/or translation of mRNAs containing AU-rich elements (AREs) in their 3'-untranslated regions. This discovery transformed our understanding of posttranscriptional gene expression. In 1997 we expanded our studies into the field of cytokine gene expression in monocytes, as many mRNAs encoding immune regulatory proteins harbor AREs. We and others have recently discovered that AUF1 and other ARE-binding proteins often act in concert with microRNAs. This has led to the merging of these two fields.

Over the past several years, we have moved into four new virus-related areas. The **first** involves examining how AUF1 and other ARE-binding proteins control IRES-dependent translation of enterovirus 71 (EV71) RNA. In collaboration with Dr. Shin-Ru Shih (Taiwan), we discovered that the RNA processing enzyme, Dicer, generates small RNAs from double-stranded regions of an IRES; these small RNAs control translation of viral RNAs. One of our long-term goals is to examine how interactions between the small, virus-derived RNAs, vsRNAs, and RNA-binding proteins control EV71.

This goal fits well with our **second** new area of investigation: linking vsRNAs, RNA-binding proteins, and EV71 IRES structure to understand how this molecular network tunes virus translation and replication. This is a collaboration with Dr. Blanton Tolbert (Case Western), who is an expert in RNA-protein structure. The Brewer/Tolbert collaboration led to a **third** area involving a collaboration with Dr. Amanda Hargrove (Duke University). Dr. Hargrove is an expert in RNA-targeted, small molecules. Together, we recently identified a small molecule that binds the EV71 IRES to promote binding by host protein AUF1, an IRES repressor; this reduces virus translation and virtually blocks virus replication in a drug concentration-dependent manner.

A **fourth** area of investigation arose due to the overwhelming COVID-19 global pandemic, caused by the coronavirus SARS-CoV-2. We are building upon the synergies between the Brewer (functional characterization of RNA-binding proteins and virus biology), Tolbert (virus RNA structural biology), and

Hargrove (RNA-targeted small molecules) labs to address SARS-CoV-2 gene expression and screen for antivirals using RNA-focused, small molecule libraries. Together with an international team, we identified small, RNA-targeted molecules that bind select stem-loops in the 5'-end of SARS-CoV-2 RNA. Reporter mRNA assays employing the firefly luciferase open read frame linked to the 5' RNA sequences showed that the lead small molecules reduce reporter luciferase activity in a concentration-dependent manner, and virus replication comparably to remdesivir. Employing the combined expertise of the Tolbert and Brewer/Li labs, we strongly believe the research proposed here will lead to new insights into enterovirus-host interactions and possibly new antiviral drugs.

Ongoing and recently completed projects that I would like to highlight include:

Ongoing:
R01 GM126833-04
Brewer, Tolbert (co-PD/PIs)
01/01/2018 – 12/31/2022
Defining the Structural Mechanisms of RNP Complexes that Regulate Enterovirus Translation
**Renewal A1 is in preparation**

Completed:
R01 GM111959
Brewer (yrs 3-4), Patel (co-PD/PIs)
09/15/2014 – 05/31/2019
Structural and Mechanistic Studies of Self and Non-Self Recognition by RIG-I

New Jersey Health Foundation PC 135-22
Brewer (PI)
02/15/2022 – 02/14/2023
Antiviral Mechanism of Novel Small Molecules that Target SARS-CoV-2 RNA Structures

Citations:


1. Calderon, J.D., Patwardhan, N., Chiu, L-Y., Sugarman, A., Cai, Z., Penutmutchu, S.R., Li, M-L., **Brewer, G.**, Hargrove, A.E., and Tolbert, B.S. 2020. Small molecule targeting IRES domain inhibits enterovirus 71 replication via an allosteric mechanism that stabilizes a ternary complex. *Nature Comm.* **22**: 4775.
2. Zafferani, M., Haddad, C., Luo, L., Davila-Calderon, J., Yuan-Chiu, L., Shema Mugisha, C., Monaghan, A.G., Kennedy, A.A., Yesselman, J.D., Gifford, R.R., Tai, A.W., Kutluay, S.B., Li, M.L., **Brewer, G.**, Tolbert, B.S., and Hargrove, A.E. 2021. Amilorides inhibit SARS-CoV-2 replication in vitro by targeting RNA structures. *Sci. Adv.* **7**: eab;6096.
3. Li, M.L., Ragupathi, A., Patel, N., Hernandez, T., Magsino, J., Werlen, G., **Brewer, G.**, and Jacinto, E. The RNA-binding protein AUF1 facilitates Akt phosphorylation at the membrane. *J. Biol. Chem.* **298:** 102437. ***Selected as an *Editors' Pick***
4. Mackeown, M., Kung, Y.A., Davila-Calderon, J., Ford, W.P., Luo, L., Henry, B., Li, M.L., **Brewer. G.**, Shih, S.R., and Tolbert, B.S. 2023. The 5'UTR of HCoV-OC43 adopts a topologically constrained structure to intrinsically repress translation. *J. Biol. Chem.* Feb 15:103028, in press. Online ahead of print


## B. Positions, Scientific Appointments, and Honors

### Positions and Scientific Appointments

| | |
|---|---|
| 2020-2023 | co-Editor (with Dr. Gerald Wilson), book entitled *RNA-based Mechanisms in Cancer* |
| 2019-2020 | co-Guest Editor (with Drs. Blanton Tolbert and Mei-Ling Li), *Methods: Methods to characterize virus small RNAs and RNA structures* |
| 2014-2018 | MID-1 Study Section, Appointed Member |
| 2012 | Guest editor, *Methods: MicroRNAs* |

| | |
|---|---|
| 2012 | Visiting Professor, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China |
| 2010-2018 | Interim Chairman, Department of Biochemistry & Molecular Biology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ |
| 2010 | Visiting Professor, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China |
| 2007 | Guest editor, *Methods: MicroRNAs Part A*, vol. 43, no. 2 |
| 2007-2011 | MGB Study Section, Appointed Member |
| 2005 | MGB Study Section *ad hoc* |
| 2004-present | Professor, Department of Biochemistry & Molecular Biology*, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ<br>(*formerly named the Department of Molecular Genetics, Microbiology & Immunology) |
| 2003 | NIH *ad hoc* reviewer for CDF-1 Study Section |
| 2002 | NIH *ad hoc* reviewer for CDF-1 Study Section |
| 2000-present | Member, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ |
| 1999 | NIH *ad hoc* reviewer for SBIRs |
| 1999-2004 | Associate Professor, Department of Molecular Genetics, Microbiology & Immunology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ |
| 1997-1999 | Associate Professor, Wake Forest Univ. School of Medicine, Winston-Salem, NC |
| 1991-2000 | American Cancer Society Study Section on Genetic Mechanisms in Cancer |
| 1989-1997 | Assistant Professor, Department of Microbiology & Immunology, Wake Forest Univ. School of Medicine |

**Honors**

| | |
|---|---|
| 2002 | FASEB MARC Travel Award to ABRCMS in New Orleans, LA; |
| 1984 | Tumor Biology Postdoctoral Training Grant, NIH |
| 1981 | Cellular and Molecular Biology Predoctoral Training Grant, NIH |

## C. Contributions to Science

1. My first major discoveries were during my postdoctoral years in which Jeffrey Ross' lab was working to examine the biochemistry of mammalian mRNA degradation. It was (finally) appreciated that mRNA stability is a major determinant of mRNA abundance, but there were no *in vitro* systems to identify the enzymes and cofactors required for selective degradation of mRNAs. My work as part of the Ross team helped to characterize the enzymes involved in degradation of histone mRNAs. Importantly, this body of work was the first to demonstrate that an *in vitro* system could recapitulate mammalian, cellular mRNA decay processes with high fidelity. (The joke at the time was that anybody could degrade RNA, but could you do it in a physiologically meaningful way.)
    a. Ross, J., Peltz, S., Kobs, G., and **Brewer, G.** 1986. Histone mRNA degradation *in vivo*: the first detectable step occurs at or near the 3' terminus. *Mol. Cell. Biol.* **6**: 4362-4371.
    b. Ross, J., Kobs, G., **Brewer, G.**, and Peltz, S. 1987. Properties of the exonuclease activity that degrades H4 histone mRNA. *J. Biol. Chem.* **262**: 9374-9381.
    c. Peltz, S., **Brewer, G.**, Kobs, G., and Ross, J. 1987. Substrate specificity of the exonuclease activity that degrades H4 histone mRNA. *J. Biol. Chem.* **262**: 9382-9388.

2. The work described above led to focusing on identifying the *trans*-acting factors that control degradation of *MYC* mRNA. This was important due to the roles of Myc as a transcription factor and oncoprotein. Our *in vitro* system revealed the mRNA decay pathway (and substantiated in whole-cell experiments) and identified protein and RNA factors that contribute to its degradation in vitro. These discoveries led to our identification of the first RNA-binding protein for AU-rich elements (AREs). We referred to it as AU RNA binding and degradation factor 1, AUF1. AREs, which *MYC* mRNA contains in its 3'UTR, have become the best characterized *cis*-acting, mRNA decay elements. Many mRNAs encoding oncoproteins, cytokines, and signaling proteins harbor AREs. As such, our discovery and characterization of AUF1, and the discovery of additional ARE-binding proteins by other groups, have transformed the field of posttranscriptional control of gene expression.

a. **Brewer, G.** 1991. An A+U-rich element RNA-binding factor regulates c-*myc* mRNA stability *in vitro*. *Mol. Cell. Biol.* **11**: 2460-2466.
b. Zhang, W., Wagner, B.J., Ehrenman, K., Schaefer, A.W., DeMaria ,C.T., Crater, D., DeHaven, K., Long, L., and **Brewer, G.** 1993. Purification, characterization and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. *Mol. Cell. Biol.* **13**: 7652-7665.

3. Our cloning and characterization of AUF1 in 1993 opened the door for much fruitful collaboration with numerous investigators over the years. These have included examining the roles of AUF1 in cardiovascular disease, the immune system, and heat shock responses, among many others. We and others also discovered that AUF1 and numerous RNA-binding proteins often act in concert or antagonistically with microRNAs. This has merged two fields that, in retrospect, had to be linked somehow.
    a. Laroia, G., Cuesta, R., **Brewer, G**., and Schneider, R.J. 1999. Control of cytokine mRNA decay by the heat shock-ubiquitin-proteasome pathway. *Science* **284**: 499-502.
    b. Wu, X., Chesoni, S., Rondeau, G., Tempesta, C., Patel, R., Charles, S., Daginawala, N., Zucconi, B.E., Kishor, A., Xu, G., Shi, Y., Li, M-L., Irizarry-Barreto, P., Welsh, J., Wilson, G.M. and **Brewer, G.** 2013. Combinatorial mRNA binding by AUF1 and Argonaute 2 controls decay of selected target mRNAs. *Nucl. Acids Res*. **41**: 2644-2658.

4. An important, long unknown question was how AUF1 acts to promote ARE-mRNA degradation and how signaling pathways regulate mRNA degradation. Work performed in collaboration with Bob Schneider's lab linked AUF1 to proteasomes, control of mRNA degradation during heat shock, and complexes of AUF1 with heat shock proteins Hsp/Hsc70. We subsequently observed complexes between AUF1 and Hsp27 and that activation of MAPK signaling stabilizes numerous proinflammatory cytokine mRNAs. But we didn't know how. We revealed in a series of papers that MAPK signaling triggered degradation of AUF1 by proteasomes. In addition to phosphorylation of both Hsp27 and AUF1, mRNA stabilization requires the F-box protein β-TrCP, the substrate recognition subunit of the E3 ubiquitin ligase Skp1–cullin–F-box protein complex, $SCF^{\beta\text{-TrCP}}$. This work showed for the first time that a signaling axis composed of p38 MAP kinase–MK2–Hsp27–β-TrCP promotes AUF1 degradation by proteasomes and stabilization of cytokine ARE-mRNAs.
    a. Knapinska, A.M., Gratacós, F.M., Krause, C.D., Hernandez, K., Jensen, A.G., Bradley, J., Pestka, S. and **Brewer, G.** 2011. Chaperone Hsp27 modulates AUF1 proteolysis and AU-rich element-mediated mRNA degradation. *Mol. Cell. Biol*. **31**: 1419-1431.
    b. Li, M-L., Defren, J., and **Brewer, G.** 2013. Hsp27 and F-box protein β-TrCP promote degradation of mRNA decay factor AUF1. *Mol. Cell. Biol.* **33**: 2315-2326.

5. During the past several years, we have redirected our efforts towards examining how AUF1 and other RNA-binding proteins control IRES-dependent translation of enterovirus 71, EV71 RNA. EV71 is a positive-strand RNA virus that is the major cause of hand-foot-mouth disease in children; it can also cause severe neurological complications. The long 5'UTR harbors a type I IRES that is essential for translation and virus replication. We found that AUF1 binds stem-loop II (SL-II) within the IRES; AUF1 negatively regulates IRES activity by unknown mechanisms. The addition of a new collaborator, Dr. Amanda Hargrove (Duke), identified an amiloride-based, small molecule that binds the EV-71 SL-II, which promotes AUF1 binding; this reduces virus translation and blocks virus replication in a drug concentration-dependent manner. In response to the COVID-19 pandemic, we expanded our studies to include coronaviruses, which are also single, positive strand RNA viruses. They too depend on stem-loop structures in their 5'UTRs. Rescreening the Hargrove amiloride-based library identified three lead small molecules that bind the SAR2-CoV-2 RNA 5'-end and block virus protein synthesis. We filed a patent application Dec. 2021 for these three molecules as antivirals.

    a. Li, M-L., Lin, J-Y., Chen, B-S., Weng, K-F., Shih, S-R., Calderon J.D., Tolbert, B.S., and **Brewer, G.** 2019. EV71 3C protease induces apoptosis by cleavage of hnRNP A1 to promote apaf-1 translation. *PLoS One* **14**: e0221048.
    b. Calderon, J.D., Patwardhan, N., Chiu, L-Y., Sugarman, A., Cai, Z., Penutmutchu, S.R., Li, M-L., **Brewer, G.**, Hargrove, A.E., and Tolbert, B.S. 2020. Small molecule targeting IRES domain inhibits enterovirus 71 replication via an allosteric mechanism that stabilizes a ternary complex. *Nature Comm.* **22**: 4775.

c. Zafferani, M., Haddad, C., Luo, L., Davila-Calderon, J., Yuan-Chiu, L., Shema Mugisha, C., Monaghan, A.G., Kennedy, A.A., Yesselman, J.D., Gifford, R.R., Tai, A.W., Kutluay, S.B., Li, M.L., **Brewer, G.**, Tolbert, B.S., and Hargrove, A.E. Amilorides inhibit SARS-CoV-2 replication in vitro by targeting RNA structures. *Science Advances*, 2021; **7**: eabl6096.
d. Mackeown, M., Kung, Y.A., Davila-Calderon, J., Ford, W.P., Luo, L., Henry, B., Li, M.L., **Brewer. G.**, Shih, S.R., and Tolbert, B.S. 2023. The 5'UTR of HCoV-OC43 adopts a topologically constrained structure to intrinsically repress translation. *J. Biol. Chem.* Feb15:103028, in press.

**Complete List of Published Work in MyBibliography:**
https://www.ncbi.nlm.nih.gov/sites/myncbi/gary.brewer.1/collections/62213462/public/