
BIOGRAPHICAL SKETCH

Senior Trainer

NAME: Colin R. Jefcoate

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

POSITION TITLE: Professor of Cell and Regenerative 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	FIELD OF STUDY
Oxford University, Oxford, England	B.S.	1963	Chemistry
Oxford University, Oxford, England	Ph.D.	1966	Chemistry
Basal University, Switzerland	Postdoctoral	1966-1967	
Cornell University, Ithaca, NY	Postdoctoral	1967-1969	
Edinburgh University, Edinburgh, Scotland	Postdoctoral	1969-1972	
University of Wisconsin, Madison, WI	Postdoctoral	1972-1973	

A. Personal Statement

The theme that has developed, over time, in my laboratory is one of **environmental adaptation**: to **Diet**, to **Chemical exposure** and to **Stress**. These studies have emerged from long-time interests in P450 Cytochromes and endocrinology. **The early characterization of Cytochrome P450_{scc} (CYP11A1) led to the discovery that cholesterol transport in mitochondria was the limiting step**, which led to the identification of the Steroidogenesis Acute Regulator (StAR) by Clark and Stocco in 1994. Much subsequent work from my laboratory has gone into understanding how StAR functions in natural steroid producing cells and endocrine processes. The search for the P450 Cytochrome in mouse embryo fibroblasts led to our purification and cloning of the anomalous **Cytochrome P450 1B1 (CYP1B1)**. The motivation for this search was that this Cytochrome P450 would have novel physiological functions, in view of the multi-potential role of the cells.

The effort to bridge in vivo and in vitro problems has led to a recent emphasis on transgenic mice and systems quantitative microarray analyses for studies of diet and Cyp1b1 deficiency, to the use of spectroscopy to probe the relationship of substrate cholesterol to Cyp11a1 and, most recently, to a 5-year commitment to develop novel FISH probes that can detect single RNA molecules or gene loci in cultured cells and fixed tissues. Now as a member of a new Department of Cell and Regenerative Biology, the lab has emphasized developmental processes, notably functions of CYP1B1 in mesenchymal progenitor cells, including the bone marrow and testis.

As Director of the Molecular and Environmental Toxicology Center for over 20 years, I developed a major interest in University Education, including minority education. This has been complemented by work through the Center for Global Health on overseas training and research. In the past 5 years, I have spent a month in Wuhan China and time in Sierra Leone, teaching classes in both locations. As Center Director, I was successful in starting an NIEHS Center for Developmental Toxicology. I have remained committed to this area of research and to collaborations that foster this work

As a part of my long-standing commitment to undergraduate and graduate education, I continue to direct a course in Molecular Toxicology, which is a core course for the graduate programs in Toxicology and Pharmacology (each T32 supported) and for our outstanding 2-year undergraduate Pharmacology/Toxicology research training program. The 21st PhD student from my laboratory has defended her thesis, and I continue to offer entry level research opportunities to an average of 3 undergraduates per year. My Associate Editor work for the Journal of the National Cancer Institute keeps me on top of new developments in Cancer Etiology. I remain committed to serving the NIH review process, as an ad hoc reviewer.

The interests in Cyp1b1 and StAR, which were selected because of the breadth of their impact, are providing a wealth of challenging projects, for which we have developed numerous resources. My present priority is to transfer the novel concepts and technology to young investigators, while also invigorating the projects.

B. Positions and Honors

Positions and Employment

1966-1967	NATO Fellow, P. Hemmerich, Basal University, Switzerland
1967-1969	NIH and NATO Fellow, J.L. Gaylor, Cornell University, Ithaca, NY
1969-1972	MRC Fellow, G.S. Boyd, Edinburgh University, Edinburgh, Scotland
1972	Visiting Scientist, W.H. Orme-Johnson, Biochemistry, University of Wisconsin, Madison, WI
1972-1973	Research Associate, W.H. Orme-Johnson, Biochemistry, University of Wisconsin, Madison, WI
1973-1978	Assistant Professor, Pharmacology, University of Wisconsin, Madison, WI
1978-1982	Associate Professor, Pharmacology, University of Wisconsin, Madison, WI
1982-2011	Professor, Pharmacology, University of Wisconsin, Madison, WI
1983-1998	Director, Environmental Toxicology Center, University of Wisconsin, Madison, WI
1998-2003	Director, NIEHS Center for Developmental and Molecular Toxicology, University of Wisconsin, Madison, WI
2011-Present	Professor, Cell and Regenerative Biology, University of Wisconsin, Madison WI

Other Experience and Professional Memberships

1972	NATO Science Committee Conference on Catalysis (Italy)
1976-1978	NIH Endocrinology Study Section (Ad Hoc)
1978-1980	NIH Chemical Pathology Study Section (Ad Hoc)
1980-1983	NIH Chemical Pathology Study Section (Regular)
1986	Preclinical Cancer Program Project (Regular)
1992-1994	Molecular Biology Specialty Section, Society of Toxicology
1995-1996	Editor of <i>Physiological Functions of Cytochrome P450</i> , published October, 1996
1995	American Association for Cancer Research, Organizer of Instructional Workshop on Cytochrome P450 and Cancer
1996	International Meeting on Microsomes and Drug Metabolism, Los Angeles. Organizer of session on Chemical Carcinogenesis and Teratogenesis
2000	National Cancer Institute Study Group for Research on Genotoxicity of Estrogens
2000-2003	External Advisor, University of New Mexico NIEHS Center
2001	External Advisor, University of California-Santa Cruz Toxicology Program
2001	Annual Symposium, Department of Biochemistry, University of Buenos Aires. "Regulation of cholesterol conversion to steroid hormones"
2002	Plenary Lecturer – Japanese Society for Molecular Approaches to Public Health. University of Tokyo. "Toxicant disruption of adipocyte differentiation"
2002	Society of Toxicology, President Molecular Biology Specialty Section
2002-2003	UW Biomolecular Chemistry Committee
2003	Invited Lecturer, American Heart Association. "Mechanism of cholesterol transport"
2003	Annual Symposium of the Indian Institute of Toxicology, Lucknow. "Adipocyte differentiation as a model for developmental toxicants"
2004	Invited Lecture, American Society for Pharmacology and Experimental Therapeutics Annual Meeting. Symposium on Transgenic Mice and Drug Metabolism. "Role of CYP1B1 in bone marrow toxicity of polycyclic aromatic hydrocarbons".
2004	Society of Toxicology Elections Committee
2003	External Advisor, University of Queensland School of Biomedical Sciences
2003	Georgetown University DOD Center of Excellence for Environmental Effects on Breast Cancer
2003-2006	Associate Editor, Toxicological Sciences
2004	Advisory Committee for UW Dept. of Population Health Sciences
2004	UW Promotions Committee
2004	UW Medical School Research Committee

2004-2007	Fox Chase Cancer Center NIEHS Center for Environmental and Developmental Effects on Breast Cancer
2004-Present	Associate Editor, Journal National Cancer Institute
2006	Invited Lecturer, Society of Toxicology Annual Meeting. "TCDD effects on adipogenesis"
2010	Invited Lecturer, International Adrenal Meeting. "cAMP stimulation of StAR expression and cholesterol metabolism is modulated by co-expression of labile suppressors of transcription and mRNA turnover"
2010	Guest Lecturer, Course on Molecular Toxicology, Wuhan University, Wuhan, China
2010	Invited Lectures, Chinese Center for Disease Control and Prevention and Peking University, Beijing, China
2010	Invited Lectures, Hiroshima University, Osaka University and University of Tokyo, Japan
2012	Course development and grant award, International Programs Office, College of Agriculture and Life Sciences in collaboration with the University of Sierra Leone Fourah Bay College. Development of sustainable community-based environmental health care approaches to track and prevent waterborne disease outbreaks in Sierra Leone
2013	Guest Editor, Proceedings of the National Academy of Sciences
2015	NIH ICER Study Section (Ad Hoc)
Present	Member of the Society of Toxicology
Present	Member of American Society for Pharmacology and Experimental Therapeutics (ASPET)

Honors and Awards

1964	2 nd class Honors in Natural Science (Chemistry)
1966	D. Phil. (Chemistry) under R.O.C. Norman, Oxford (including one year at York University)
1998-2003	WARF Mid-Career Faculty Researcher Award
2013	H Beinert Professor of Biological Sciences

C. Contribution to Science

1. Fundamental steps in the control of cholesterol conversion to steroid hormones

StAR functions as the gatekeeper of steroid synthesis. The early work from this lab established that a labile protein activity transferred cholesterol into mitochondria to enhance complex formation with Cyp11a1. Complex formation was measured in cells and tissues by optical and EPR spectroscopy measurements of the cholesterol-Cyp complex (1). We showed that the cytochrome oxidized cholesterol to pregnenolone at a very high rate, mediated by a shuttling of the electron donor, ferredoxin (adrenodoxin), from the NADPH-directed reductase (2). Cholesterol metabolism was activated by cAMP in adrenal fasciculate cells by PKA, through a 37 kd labile phospho-protein identified by Orme-Johnson in adrenal fasciculate cells that we also found to be Ca-regulated in glomerulosa cells. The Stocco laboratory identified StAR as the responsible gene. StAR activation can be achieved by binding to the outer mitochondrial membrane in recombinant models. We showed that the remarkably high rates in adrenal cells (400 cholesterol/newly synthesized StAR) required constant renewal of synthesis, as well as PKA phosphorylation. In addition, import into mitochondria coupled to N-terminal cleavage was necessary for activity (3-4). Work from several labs in the past 10 years has shown that Porin-associated channels and fusion of mitochondria, as well as ER fusion-linked Ca transfer are important players in the cholesterol transfer.

1. Privalle CT, Crivello JR, and Jefcoate CR (1983) Regulation of intramitochondrial cholesterol transfer to side chain cleavage cytochrome P-450 in rat adrenal gland. *Proc. Natl. Acad. Sci. U.S.A.* **80**:702-706.
2. Hanukoglu I and Jefcoate CR (1980) Mitochondrial cytochrome P-450 scc. Mechanism of electron transport by adrenodoxin. *J Biol Chem* **255**:3057-3061.
3. Artemenko IP, Zhao D, Hales DB, Hales KH, and Jefcoate CR (2001) Mitochondrial processing of newly synthesized steroidogenic acute regulatory protein (StAR) but not total StAR, mediates cholesterol transfer to cytochrome P450 side chain cleavage enzyme in adrenal cells. *J. Biol. Chem.* **276**:46583-46596.
4. Jefcoate C (2002) High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. *J. Clin. Invest.* **110**:881-890.

2. Regulation of StAR activity

The lab has focused on the cAMP stimulation of StAR, including the formation of alternative 1.6- and 3.5-kb transcripts with differential turnover. We identified the Zn finger protein, ZFP36L1, that binds to elements in the 3.5-kb transcript as a mediator of this turnover. We expanded understanding of the role of Sik1 and CRT2 as mediators of the PKA stimulation in adrenal cortex cells, including showing their co-localization in the nucleus. ZFP36L1 and Sik1 are co-induced with StAR by hormonal activation, even though they each suppresses the activity. Recently, we have developed high resolution FISH, which has allowed us to detect primary and spliced transcripts at the StAR loci, and to characterize the gene positioning relative to the nuclear envelope (5-8). We have shown, in testis MA10 cells, that the initial transcription is activated over 60 min, as loci activate singly and cells become engaged asynchronously. This process is mediated by activation of CRT2, as SIK kinases become inhibited by PKA. SIK1, the predominant nuclear form, is induced ten-fold by PKA in 30 min, raising important questions about the role of this inhibitory kinase in StAR-mediated steroid synthesis.

1. Lee J, Foong YH, Musaitif I, Tong T, Jefcoate C (2016) Analysis of specific RNA in cultures cells through quantitative integration of q-PCR and N-SIM single cell FISH images: Application to hormonal stimulation of StAR transcription. *Mol. Cell. Endocrinol.* **429**:93-105. PMID: In Progress.
2. Lee J, Tong T, Duan H, Foong YH, Musaitif I, Yamazaki T, Jefcoate C (2016) Regulation of StAR by the N-terminal Domain and Coinduction of SIK1 and TIS11b/Znf36l1 in Single Cells. *Front. Endocrinol.* **7**:107. PMID: PMC4969582.
3. Lee J, Yamazaki T, Dong H, Jefcoate C (2017) A single cell level measurement of StAR expression and activity in adrenal cells. *Mol. Cell. Endocrinol.* **441**:22-30. PMID: In Progress.
4. Lee J and Jefcoate C (2017) Monitoring of dual CRISPR/Cas9-mediated steroidogenic acute regulatory protein gene deletion and cholesterol accumulation using high-resolution fluorescence in situ hybridization in a single cell. *Front. Endocrinol.* **8**:289. PMID: PMC5660980.

3. Identification and regulation cytochrome P4501b1 (CYP1B1)

The **identification of CYP1B1** resulted from our purification of the protein from mouse embryo fibroblasts in 1990, and the generation of antibodies used in the specific recognition of the protein in multiple tissues, and provided the means for specific cloning (9). Hormonal regulation of Cyp1b1 in the rat adrenal and testis led to cloning and establishment of SF1 regulation (10). Analysis of the Cyp1b1 promoter and the induction by Ah Receptor (AhR) activators showed that a GC-rich enhancer region contained two anomalous AhR binding elements, in addition to a canonical AhR/Arnt DRE element (11). These anomalous elements bind an inhibitory complex through sequences that overlap the DRE (12). Cyp1b1 is also anomalous by containing a 3-kb 3'UTR that includes AU-rich regulatory sites typical of labile, inflammatory gene products. **CYP1B1** is expressed in mesenchymal progenitors and other fibroblastic cells, steroid producing cells, endothelia, glia and inflammatory cells.

1. Savas Ü, Bhattacharyya KK, Christou M, Alexander DL, and Jefcoate CR (1994) Mouse cytochrome P450EF, representative of a new 1B subfamily of cytochrome P450s. Cloning, sequence determination, and tissue expression. *J. Biol. Chem.* **269**:14905-14911.
2. Bhattacharyya KK, Brake PB, Eltom SE, Otto SA, and Jefcoate CR (1995) Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. *J. Biol. Chem.* **270**:11595-11602.
3. Zhang L, Zheng W, and Jefcoate CR (2003) Ah receptor regulation of mouse Cyp1B1 is additionally modulated by a second novel complex that forms at two AhR response elements. *Toxicol. Appl. Pharmacol.* **192**:174-190.
4. Zheng W and Jefcoate CR (2005) Steroidogenic factor-1 interacts with cAMP response element-binding protein to mediate cAMP stimulation of CYP1B1 via a far upstream enhancer. *Mol. Pharmacol.* **67**:499-512.

4. Role of Cyp1b1 in toxicology and physiology

Cyp1b1 is constitutively expressed in many multi-potential mesenchymal cells, as well as tumor cell types. We assume that Cyp1b1 plays a role in important physiological functions, although it is better known for metabolism of polycyclic hydrocarbons. The lab has played a leading role in showing that Cyp1b1 mediates the **activation of several polycyclic aromatic hydrocarbons (PAHs)** to reactive metabolites that participate in PAH induced carcinogenesis. Cyp1b1 is expressed in bone marrow multi-potential stromal cells, which mediate PAH

suppression of hematopoietic progenitors (13-14). We have recently repeated this selective targeting in a co-culture system of mesenchymal support cells and bone marrow primary cells.

CYP1B1 controls vascular adhesion, monocyte differentiation and potentially modulates differentiation of mesenchymal progenitors or local stromal support processes. A collaboration with Dr. Nadar Sheibani's lab has shown that Cyp1b1 deletion in endothelia and pericytes induces oxidative stress, while also changing cell adhesion and releasing active matrix proteins. Deletion in humans causes congenital glaucoma, in part through loss of matrix regulation by Cyp1b1 in mesenchymal trabecular meshwork cells.

The appreciable Cyp1b1 expression in mouse bone marrow stroma may play a role in osteogenic, adipogenic and hematopoietic differentiation processes. Expression in adipogenesis parallels that of PPAR γ , but is mechanistically uncoupled from the differentiation process, which we showed to be sensitive to AhR-directed processes. We have recently showed that Cyp1b1 deletion, *in vivo*, lowers diet-induced adiposity in conjunction with extensive diet-sensitive changes in liver gene expression (15-16). This suppression is tightly coupled to Cyp1b1-dependent effects on GH-Hnf4a signaling, which undergoes a major transition as the mice reach maturity. Current work addresses the hypothesis that the Cyp1b1 relationship with GH depends on neonatal metabolism of estradiol in the hypothalamus, which establishes adult neuroendocrine regulation of the liver.

1. Larsen MC, N'jai AU, Alexander DL, Rondelli CM, Forsberg EC, Czuprynski CJ, and Jefcoate CR (2016) Cyp1b1-mediated suppression of lymphoid progenitors in bone marrow by polycyclic aromatic hydrocarbons coordinately impacts spleen and thymus: a selective role for the Ah receptor. *Pharma. Res. Per.* **4**: e00245 PMID: PMC5242170.
2. Rondelli CM, Larsen MC, N'jai A, Czuprynski CJ, Jefcoate CR (2016) PAHs Target Hematopoietic Lineages in Bone Marrow through Cyp1b1 Primarily in Mesenchymal Stromal Cells but Not AhR: A Reconstituted In Vitro Model. *Stem Cells Int.* 2016:1753491. Epub 2017 Nov 7. PMID: PMC5116507.
3. Larsen MC, Bushkofsky JR, Gorman T, Adhami V, Mukhtar H, Wang S, Reeder SB, Sheibani N, Jefcoate CR (2015) Cytochrome P450 1b1: An unexpected modulator of liver fatty acid homeostasis. *Arch. Biochem. Biophys.* **571**:21-39. PMID: PMC4387869.
4. Bushkofsky, JR, Maguire M, Larsen MC, Foong YH and Jefcoate CR (2016) Cyp1b1 affects external control of mouse hepatocytes, fatty acid homeostasis and signaling involving HNF4 α and PPAR γ . *Arch. Biochem. Biophys.* **597**:30-47. PMID: PMC4868071.

D. Research Support

Ongoing Research Support

R01 HD09090660 Jorgensen (PI), Jefcoate (co-PI)
NICHD

08/16/2017-04/30/2022

Mediators for dynamic regulation of Star transcription is conserved across fetal and adult Leydig cells.

Major goal: Examine the role of StAR in juvenile and adult testosterone synthesis and regulation in the testes.

Previous Research Support

R01 DK090249 Jefcoate (PI)
NIH

04/01/2012-03/31/2017

Cytochrome P4501B1 and basal liver PPAR α activity

Major goal: (1) Understand how Cyp1b1 metabolism can impact endogenous ligands for CAR and PPAR activities in hepatocytes, even though Cyp1b1 is not expressed in these cells; (2) Develop a floxed Cyp1b1 mouse. This mouse will be used to derive conditional transgenic mice that have cell- or time-selective deletions of Cyp1b1.