BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
David M. Gilbert	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) gilbertdm	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
U.C. San Diego, La Jolla, CA.	B.A.	05/82	Biochemistry/Cell Biol.
Stanford University, Stanford, CA	Ph.D.	11/89	DNA Replication
Faculté de Médecine, Strasbourg, FRANCE	Post-Doc	11/91	Chromatin/Transcription
Roche Institute of Mol. Biol., Nutley, N.J.	Post-Doc	08/94	DNA Replication

A. Personal Statement

I have a broad background in cell and molecular biology, particularly as it pertains to DNA replication, chromatin structure, transcription and nuclear organization. As a graduate student with Stanley Cohen, I pioneered studies of differential replication timing and its relationship to gene expression (e.g. PNAS, 1986: **83**, 2924). I developed the use of anti-BrdU antibodies to study replication prior to their commercialization by BD, and I developed the FACS-based method to analyze replication timing that is currently used for almost all systems. I then broadened my perspective by studying transcriptional control and chromatin structure as a post-doc with Pierre Chambon, followed by a second post-doc with Melvin DePamphilis studying replication origins in mammals, where I developed the first cell-free system to study cell cycle control of replication origin selection. I made seminal contributions to each of these fields, and have always strived to shift paradigms rather than contribute to the status quo. As PI on several NIH-funded grants, I discovered two novel G1-phase control points when replication timing and origin site selection are determined. These discoveries revealed links between replication control and large-scale chromatin reorganization in the newly formed nucleus. Later, I expanded my research to study developmental control of replication timing using embryonic stem cell differentiation systems, systematically characterizing where and when differentiation-induced changes in replication timing take place during early mouse and human development, and how these changes relate to changes in transcription and sub-nuclear organization of chromatin during cell fate changes. I now maintain the largest worldwide database of RT in different cell types (replicationdomain.org). Using integrative genomics methods, in collaboration with ENCODE, we demonstrated that topologically associating structural domains of chromosomes, predicted computationally from Hi-C data, are functional units of replication timing developmental regulation that self-organize in early G1 phase, coincident with the establishment of a replication timing program. These findings have provided a unified view of large-scale chromosome structure and function. Recently, using genome engineering approaches, we identified *cis*-acting elements regulating replication timing that have opened the door to mechanistic understanding of replication timing and its enigmatic relationship with transcription and 3D chromosome architecture. In summary, I have a demonstrated record of successfully directing innovative research projects that address complex problems of large-scale chromosome structure and function during the cell cycle and development. With specific regard to the proposed experiments, I have a demonstrated record of successful collaboration to leverage emerging technologies and I am a member of the 4D Nucleome consortium. Combined with my background, I believe that I am well positioned to put the proposed technologies into action to address critical questions regarding the 3D architecture of human cellular DNA replication, an understudied essential function of our genomes.

B. Positions and Honors

Professional Positions

9/94-6/98 Assistant Professor, SUNY Health Science Center at Syracuse (SUNY HSC)
6/98-4/03 Associate Professor, SUNY Upstate Medical University (formerly SUNY HSC)
4/03–8/06 Full Professor, SUNY Upstate Medical University
8/06-Present J. Herbert Taylor Distinguished Professor of Molecular Biology, Florida State University

4/15-Present Distinguished Research Professor, Florida State University
1/15-Present Co-founder of the Center for Genomics and Personalized Medicine

Awards and Other Professional Activities

1989-1991 Eur. Mol. Biol. Org. (EMBO) Post-Doctoral Fellowship
1990 NATO Post-Doctoral Fellowship (declined)
1991-1994 Roche Post-Doctoral Fellowship
1995-1998 Peer Review for the US Army Reserve Medical Corps Breast Cancer Program
1997-Present Peer Review for NIH
1996-2004 Peer Review for the American Cancer Society
2002 SUNY Upstate President's Award for Excellence in Research for a Young Investigator
2004 Nominated as a Howard Hughes Medical Institute Investigator
2004-2005 NIH Career Enhancement (K18) Award for Stem Cell Research
2004-2006 Selected to compete in Phase II of the NIH Director's Pioneer Award (NDPA)
2008-Present Board Member, SouthEast Stem Cell Consortium (SESCC)
2008-Present Board Member, Epigenetics Society
2008-Present Editorial board; Journal of Cell Biology
2008-Present Elected Fellow of the AAAS
2010-Present Elected Council Delegate of the AAAS, section on Biological Sciences
2011-Present Member of NIH ENCODE2, ENCODE3 and mouseENCODE consortia
2013-Present Elected member of the American Society for Hematology (ASH)
2014-Present Member International Society for Stem Cell Research (ISSCR)
2015 Florida State University's Distinguished Research Professor award.
2015-2018 FSU Biology Pfeiffer Endowed Professorship for Cancer Research
2016-2019 Co-Chair and Chair of the GRC meeting on Genome Architecture and Disease
2016 Florida State University Graduate Faculty Mentorship Award
2016- Present Co-Chair of the NIH 4D Nucleome Consortium Cell Lines and Samples WG

C. Contributions to Science

1. Mammalian Replication Origin Specification and the Origin Decision Point (ODP): The mechanisms by which multi-cellular organisms select replication initiation sites remain a mystery. For decades it was assumed that specific DNA sequences function as defined replicators but attempts to identify such sequences failed and several systems were found to initiate efficiently with no sequence specificity. I developed the first cell-free system that could initiate eukaryotic replication site-specifically requiring chromatin derived from cells synchronized after a specific time during G1 phase, termed the Origin Decision Point (ODP). Initiation within chromatin from early G1 phase nuclei was equally efficient, but occurred with no site-specificity. We demonstrated that the ODP is upstream of the restriction point and that transcription can focus initiation to intergenic regions. By perturbing cell cycle checkpoints, we could confirm this finding in living cells, and showed that failure to select origins at the ODP elicits a checkpoint arrest that is abrogated in transformed cells This work changed the way we think about origins from defined sequences to flexible entities whose usage is governed by complex contextual features and activities, and in doing so, unified a seemingly irreconcilable literature. In 2001, I wrote a review article ("Making Sense of Eukaryotic Replication Origins") that I believe summed up the situation in a manner that has not changed because of the stochastic nature of initiation in mammalian cells. In unpublished work with Nick Rhind (U. Mass), we have developed a single molecule method ("Optical Replication Mapping; ORM) that *will vertically transform the field* by being capable of measuring the frequencies of usage of different origins and their coordination on the same DNA molecules at high throughput, which is certain to draw me back into this field.

a. Wu, J.W. and **Gilbert, D. M.** (1996) A Distinct G1 Step Required to Specify A Mammalian Replication Origin **SCIENCE** 271: 1270-1272.
b. Wu, J.-W. and **Gilbert, DM** (1997) The Replication Origin Decision Point is a Mitogen Independent, 2-Aminopurine Sensitive, G1-Phase Event That Precedes Restriction Point Control. **MOL. CELL. BIOL.**17: 4312-4321.
c. Wu, J.-W. and **Gilbert, D.M.** (1998) Transformation Abrogates an Early G1-Phase Arrest Point Required for Specification of the Chinese Hamster DHFR Replication Origin **EMBO J.** 17: 1810 – 1818
d. Takayo Sasaki, Sunita Ramanathan, Yukiko Okuno, Chiharu Kumagai, Seemab S. Shaikh and **Gilbert**, **D.M.** (2006) The Chinese Hamster DHFR Replication Origin Decision Point Follows Activation of Transcription and Confines Initiation to the Intergenic Region, **MOL. CELL. BIOL.** 26:1051-1062.

2. Pre-RC Protein Assembly and Dynamics: Critical to genome integrity is the need to duplicate the genome exactly once during each S phase. This is executed by two mutually exclusive periods of the cell cycle; one in

which pre-replication complexes (pre-RCs) can form but replication cannot initiate, and a second during which replication can initiate but pre-RCs cannot re-form. During the 1990s, studies in budding yeast and *Xenopus* egg extracts identified the factors controlling the first step, pre-RC assembly, which culminates in the loading of two heterohexameric Mcm2-7 helicase complexes. My group pioneered studies of pre-RCs in mammalian cells. We were the first to demonstrate that pre-RCs form shortly after activation of the anaphase promoting complex, much earlier in the cell cycle than expected. To date we are the only group to demonstrate that the physical association of Mcm2-7 with chromatin during telophase represents the *functional* assembly of pre-RCs, by demonstrating that *Xenopus* egg extracts lacking pre-RC proteins or assembly activities can initiate replication efficiently with isolated telophase, but not metaphase, chromatin. We are also the only group to demonstrate using an unbiased approach that pre-RCs are assembled near the sites where replication will initiate. Finally, we showed that loading of the Mcm helicase during telophase is static, unlike any other chromatin protein. Its off rate is undetectable, even under conditions of prolonged cell cycle arrest, requiring DNA replication for eviction in an unprecedented "lockdown/kickoff" manner. These studies have formed a foundation on which future origin and pre-RC protein mapping studies will build when appropriate technical hurdles are surpassed. Our live cell Mcm imaging studies also have contributed to our view of chromatin biology in living cells, demonstrating that not all chromatin proteins are dynamic.

a. Dimitrova, D.S., Todorov, I.T., Melendy, T. and **Gilbert, D.M.** (1999) Mcm2, But Not RPA, is a Component of the Mammalian Early G1-Phase pre-Replication Complex. **J. CELL BIOL.** 146: 1-14
b. Okuno, Y., McNairn, A.J., den Elzen, N., Pines, J. and **Gilbert, D.M.** (2001) Stability, Chromatin-Association and Functional Activity of Mammalian Pre-Replication Complex Proteins During the Cell-Cycle, **EMBO J.** 20: 4263-4277
c. Lubelsky, Y, Sasaki, T., Kuipers, M.A., Lucas, I., Le Beau, M.M., Carignon, S., Debatisse, M., Prinz, J.A., Dennis, J. H. and Gilbert, D.M. (2011) Pre-replication complex proteins assemble at regions of low nucleosome occupancy within the Chinese hamster dihydrofolate reductase initiation zone, **NUCL. ACIDS RES.,** 39:3141-3155 (PMC3082903)
d. Kuipers, M.A., Stasevich, T.J., Sasaki, T., Wilson, K.A., Hazelwood, K.L., McNally, J.G., Davidson, M.W. and **Gilbert, D.M.** (2011) Highly stable loading of Mcm proteins onto chromatin in living cells requires replication to unload. **J CELL BIOL.** 192:29-41. (PMC3019549)

3. The Replication Timing Decision Point (TDP): All eukaryotic cells replicate their DNA in a distinct temporal order (replication timing). Early cytogenetic studies by my group and others demonstrated that DNA synthesis takes place in discrete punctate foci and that foci replicating at different times during S phase are located in distinct compartments of the nucleus. Using the cell-free system described above, my lab demonstrated that the replication-timing program is established coincident with re-positioning and anchorage of these foci within the nucleus shortly after nuclear re-assembly, a time we called the Timing Decision Point (TDP). The TDP precedes the ODP and several manipulations could uncouple proper replication timing from any particular pattern of replication origin usage. We later demonstrated that determinants for replication timing are lost during S phase, despite maintenance of the 3D organization of chromatin. We proposed that anchorage of chromatin could create scaffolds that seed the assembly of sub-nuclear compartments of different molecular composition, a model that has become popular to explain many structure-function relationships in the nucleus and is conceptually similar to the popular concept of "phase separation".

a. Dimitrova, D.S. and **Gilbert, D. M.** (1999) The spatial position and replication timing of chromosomal domains are both established in early G1-phase. **MOL. CELL** 4: 983-993
b. Li, F., Chen, J., Izumi, M., Butler, M.C., Keezer, S. M. and **Gilbert, D.M.** (2001) The Replication Timing Program of the Chinese Hamster □-Globin Locus is Established Coincident With its Association With Peripheral Heterochromatin in Early G1-Phase, **J. CELL BIOL**. 154: 283-292
c. Wu, R., Singh, P.B. and **Gilbert, D.M.** (2006) Uncoupling Global and Fine-Tuning Replication Timing Determinants for Mouse Peri-Centric Heterochromatin. **J. CELL BIOL.,** 174: 185-194.
d. Lu, J., Li, F., Murphy, C.S., Davidson, M.W. and **Gilbert, D.M.** (2010) G2-Phase Chromatin Lacks Determinants of Replication Timing. **J. CELL BIOLOGY**, 89 955-965 (PMC2886351)

4. Developmental Control of Replication Timing in Stem Cells and Aberrations in Disease: Inspired by differential replication timing of genetically identical X chromosomes in female mammals, I was the first to demonstrate differential replication timing of active and inactive copies of autosomal genes. Although some genes replicate at different times in different cell lines, it could not be determined whether these were developmental differences or cell line/genomic aberrations. A dynamic system for cell fate changes was necessary. Once it became clear that embryonic stem cells (ESCs) could be differentiated to specific cells types,

I initiated work with ESCs, resulting in the first demonstration that dynamic changes in replication timing can indeed occur during differentiation. Shortly thereafter, we developed a protocol to profile replication-timing genome wide in any proliferating cell type. Coupled with transcriptome analyses in myriad differentiation schemes and mutant cells, we demonstrated that close to half the genome changes replication timing and a significant subset (not all) of genes change transcriptional state coordinately with replication timing. We found that replication timing is robust to many gene deletions and highly conserved across polymorphic individuals and to a lesser extent across species. We discovered a spatio-temporal consolidation of chromatin into larger coordinately regulated units that occurs during stem cell differentiation. We identified a set of genes that are early replicating uniquely in pluripotent cells and unusually resistant when reprogramming differentiated cells to the pluripotent state, consistently remaining silent and late replicating in cells that fail to undergo the final steps of reprogramming. We initiated studies in cancer cells, demonstrating that individual patient leukemia samples display unique replication timing profiles that can be used to sub-stratify patients and that the differences that distinguish patient samples from each other are the same as the developmentally regulated changes that distinguish normal cell types. We also used a disease in a dish approach to identify novel replication timing markers of premature aging diseases. We maintain the largest database of replication timing data (replicationdomain.org). Together, this body of work has provided a watershed of information that has upheld or refuted longstanding hypotheses about replication timing and generated many new hypotheses that are now testable with the systems that we have developed.

a. Hiratani, I, Ryba, T, Itoh, M. Yokochi, T., Schwaiger, M., Chang, C-W, Lyou, Y. Townes, T.M., Schubeler, D. and **Gilbert, D.M.** (2008) Global Re-organization of Replication Domains During Embryonic Stem Cell Differentiation **PLoS BIOLOGY** 6: e25. (PMC2561079)
b. Ryba, T., Battaglia, D, Chang, B.H., Shirley, J.W., Buckley, Q., Pope, B.D., Devidas, M., Druker, B.J., **Gilbert, D.M.** (2012) Abnormal Developmental Control of Replication Timing Domains in Pediatric Acute Lymphoblastic Leukemia. **GENOME RESEARCH**, 22: 1833-1844. (PMC3460179).
c. Rivera-Mulia, J.C., Buckley, Q., Sasaki, T., Zimmerman, J., Didier, RA, Nazor, K., Loring, JF, Lian Z, Weissman, S., Robins, AJ, Schulz, TC, Menendez, L., Kulik, MJ, Dalton, S., Gabr, H., Kahveci, T., **Gilbert, DM** (2015) Dynamic changes in replication timing and gene expression during human pluripotent stem cell differentiation and lineage specification, **GENOME RESEARCH**, Aug;25(8):1091-103. doi: 10.1101/gr.187989.114. (PMC4509994)
d. Rivera-Mulia, J.C., Desprat, R. Trevilla-Garcia, C., Cornacchi, D., Schwerer, H., Sasaki, T., Sima, J. Fells, T., Studer, L., Lemaitre, J-M, **Gilbert, D.M.** (2018) DNA replication timing alterations identify common markers between distinct progeroid diseases, **PNAS** 114: 10972-10980 (PMID 29196523)

5. The Replication Domain Model and the Discovery of Early Replication Control Elements (ERCEs): What I hope to be my most memorable contribution is the concept of domain-level regulation of replication, what we call the “replication domain model”. I have been thinking about chromosome function in terms of domain-level regulation for my entire career. Our early work showed that replication foci (discussed above) are stable units of chromosome structure consisting of multiple coordinately activated replicons that retain punctate replication pulse label for many cell generations. We showed that replication foci are sites of replication protein assembly and disassembly in a temporal sequence that can be uncoupled from DNA synthesis itself. We found that foci in different compartments have different chromatin composition and proposed that, since chromatin is assembled at the replication fork, organization of the genome into coordinately replicated domains could facilitate rapid domain-wide chromatin changes. Using genomics, we discovered that replication timing consistently changes in units of 400-800 kb, defining molecular coordinates for replication domains. We showed that domain boundaries are functionally relevant in that they can confine the effects of rearrangements. We demonstrated that replication domains correspond to topologically associating domains measured by Hi-C and their higher order folding corresponds to replication timing such that domains that contact each other replicate at similar times, forming separate sub-nuclear chromatin compartments. All of this 3D organization is re-established coincident with the TDP (above), when replication timing is established. In what I expect to be *my biggest contribution to science*, our recent unpublished work has identified *cis*-acting elements (ERCEs) regulating replication timing and 3D architecture, giving us our first handle into the molecular dissection of mechanisms regulating this large-scale chromosome structure-function liaison. In summary, our work shows that the way the genome is organized in 3D space has important functional consequences and provides a unifying model for large scale genome organization.

a. Dimitrova, D.S. and **Gilbert, D.M.** (2000) Temporally Coordinated Assembly/Disassembly of Replication Factories in the Absence of DNA Synthesis, **NATURE CELL BIOLOGY** 2: 686-694

b. Ryba, T., Hiratani, I., Lu, J., Itoh, M., Kulik, M., Zhang**, J.,** Dalton, S. and **Gilbert, D.M.** (2010) Evolutionarily conserved replication timing profiles predict long range chromatin interactions and distinguish closely related cell types. **GENOME RESEARCH** 20:761-770 (PMC2877573).
c. Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, Vera DL, Wang Y, Hansen RS, Canfield TK, Thurman RE, Cheng Y, Gulsoy G, Dennis JH, Snyder MP, Stamatoyannopoulos JA, Taylor J, Hardison RC, Kahveci T, Ren B & **Gilbert DM.** (2014) Topologically-associating domains are stable units of replication-timing regulation. **NATURE** 515: 402–405. (PMC4251741)
d. Dileep, V., Ay, F. Sima, J., Vera, DL, Noble, WS, **Gilbert, DM.** (2015) Topologically-associating domains and their long-range contacts are established during early G1 coincident with the establishment of the replication timing program. **GENOME RESEARCH**, Aug;25(8):1104-13. doi: 10.1101/gr.183699.114. Epub 2015 May 20. (PMC4509995)

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/david.gilbert.1/bibliography/40336158/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

P01 GM085354 Dalton (PI) 7/1/14 – **2/28/19**
"Understanding Mechanisms of hESC Self-Renewal and Cell Fate Commitment"
Principle Investigator: Stephen Dalton, PhD
The objective of my subproject of this PPG is to determine the order in which changes in correlated chromosome properties occur and their interdependency during the first cell cycles after stimulating human embryonic stem cell (hESC) differentiation. ***NOTE: This project will not be renewed.***

R01 GM083337 Gilbert (PI) 6/15/16 - 3/31/20
"cis-Acting Elements regulating developmental control of replication timing"
This project is to develop chromosome-engineering methods in mouse ESCs to identify cis-acting DNA/chromatin elements regulating replication timing.

U54 DK107965 Belmont (PI) 9/28/15-7/31/20
"Combined Cytological, Genomic, And Functional Mapping Of Nuclear Genome Organization"
My group's part in this technology-driven 'NIH 4D Nucleome Consortium" center grant includes developing high-resolution replication timing mapping methods, novel strategies for manipulation of large genomic regions to investigate large scale chromosome activities, and single live-cell assays for replication timing to investigate intrinsic and extrinsic variation in the replication timing program.

R21 HG010403 Gilbert (PI) 9/6/18-8/31/20
"Comprehensive mapping of pre-replication complex protein binding sites in single human cells"
In this R21, we assess the feasibility of two novel methods (ChILT and Cut & Run) to produce single cell or at least low cell number comprehensive pre-RC protein maps in mammalian cells.

Research Support Completed During the Last Three Years

R01 GM083337-05 Gilbert (PI) 2/13/12 - 12/31/15 (renewed)
"cis-Acting Elements regulating developmental control of replication timing"
This proposal was to identify the boundaries of replication domains and develop chromosome-engineering methods in mouse ESCs to identify *cis*-acting DNA/chromatin elements regulating replication timing.

R21 CA161666-01A1 Gilbert (PI) 12/7/12-11/31/15
"Replication Profiling as a diagnostic tool in B-cell Acute Lymphoblastic Leukemia"
We proposed to link unique features of the replication-timing program to ALL patient outcome by analyzing replication-timing profiles from patient cohorts with known outcomes that lack strong prognostic features.