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FELLOWS NEWSLETTER

The Fellows Newsletter is published monthly by the Office of Education, Division of Intramural Research, National Heart, Lung, and Blood Institute and distributed to NHLBI DIR members to promote the interest of DIR Fellows.

Office of Education, DIR, NHLBI
Herbert M. Geller, Ph.D., Director
Angela N. Theofilos, Program Coord.
Aurora J. Taylor, Program Coord.

DIREducation@nhlbi.nih.gov
Building 10, Room 6N248
Tel: 301-451-9440

The Science Beat
Zhiyun Ge, LRB
Dinari Harris, LMCI

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From the Director of the Office of Education

We hope you all had both a productive and enjoyable summer. I especially wanted to thank all of you who mentored Summer Interns. They clearly had a great time this summer, and the level of achievement at summer poster day was impressive. The Summer Internship program has been a huge success in attracting students to a research career, and we hope that this summer's crop will be similarly influenced.

The Office of Education welcomes two new contributors to the newsletter: Drs. Zhiyun Ge and Dinari Harris, who will be writing the "Science Beat" column for the newsletter. This column highlights the achievements of NHLBI fellows and we look forward to their future contributions. We also have several new members of the Fellows Advisory Committee: Drs. Chad Williamson and Justin Wilson. As you all know the FAC is the "eyes and ears" of the Office of Education. They keep me abreast of potential issues that fellows face within DIR, and are intimately involved in planning social and professional activities for DIR fellows, such as the annual NHLBI DIR Scientific Retreat. Participation in the FAC provides you with experience working in a collaborative environment, and so I invite all interested fellows to join. We meet on the 2nd Monday of each month.

Why do I Have an Annual Progress Report?

By Herbert Geller, Ph.D.

Each year, every NHLBI Fellow submits a progress report to accompany their fellowship renewal paperwork. Why do you need to do this? What benefit does this have beyond words on a piece of paper? Who actually reads it?

While it may seem that this is a pro-forma requirement for renewal of your fellowship, a well-crafted Progress Report has many benefits, for several different reasons. The first benefit is that it identifies your career goal. That's why the first question is simple: what are your career goals? The answer should indicate what kind of job do you want next: do you want to stay in the lab to a Principal Investigator or a Staff Scientist at NIH or a scientist in Industry? Or do you want to leave the lab for another career such as Patent Lawyer, Technical Services Representative, or in the NIH Extramural Program? Or are you still undecided? Sometimes fellows conflate their scientific goals with their career goals. In these situations, the question arises as to whether they are simply drifting along doing experiments without paying attention to the

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THE SCIENCE BEAT

By Zhiyun Ge Ph.D.

Sarkar, S. K., Marmer, B., Goldberg, G., & Neuman, K. C. (2012). Single-Molecule Tracking of Collagenase on Native Type I Collagen Fibrils Reveals Degradation Mechanism. *Current Biology22*, 1047-1056

Collagens are the most abundant proteins in the human body and are the main components of the extracellular matrix (ECM). Various cellular activities, such as angiogenesis, wound healing, tumor progression and metastasis, require the degradation of the ECM, due to the extensive tissue remodeling occurring during those processes. Matrix metalloproteases (MMPs) are a family of zinc-dependent endopeptidases responsible for the degradation of extracellular matrix components, including collagens. The collagen fibril consists of three polyproline II-like chains, staggered by one residue and coiled to form a triple helix. This unique molecular structure grants collagenextraordinary mechanical strength and broad resistance to proteolytic enzymes. Although the hydrolysis of triple-helical collagen monomers has been extensively studied, traditional ensemble kinetics analysis has been proven to be inadequate in elucidating the molecular mechanism of collagen degradation, due to the fact that native collagen fibrils are insoluble, heterogeneous, and extended substrates. Novel approaches were developed in recent years to study collagen degradation. Fluorescence correlation spectroscopy (FCS) was used to measure the diffusion of MMPs and led to the "burnt-bridge" model, where collagen proteolysis causes biased motion of MMP diffusion. However, the mechanistic details of fibrillar collagen degradation remain largely unknown.

In this study, Sarkar *et al.*tracked the trajectories of single molecules of fluorescently labeled MMP1 on collagen fibrils using a prism-type total internal reflection fluorescence microscope (TIRFM). Native collagen fibrils were immobilized on a surface and fluorescently labeled MMP1 proteins were added. The motion of single MMP1 molecules along the fibrils was monitored in real time. The results show that MMP1 diffuses along the fibrils. The diffusion, however, is not continuous and is interrupted by long pauses. A t test-based "step-finder" analysis of the trajectories of single MMP1 molecules revealed that MMP1 spends ~ 90% of the time paused on collagen fibrils. The dwell time distribution of MMP1 suggests two kinetically distinct pause states. Class I pause features a single exponential distribution and occurs randomly on the fibril, while Class II pause is consistent with a gamma distribution, indicating that MMP1 escapes these pauses via multiple sequential kinetic steps of comparable rates.

Another interesting observation the authors made was that MMP1 undergoes cleavage dependent biased and hindered diffusion, where motion towards one side of the cleavage site is favored and crossing of the cleavage sites by subsequent enzymes is hindered. Further investigation of the motion of MMP1 molecules immediately after escaping from Class II pause revealed that the initial increase in speed and displacement of MMP1 is consistent with the biased motion resulting from collagen cleavage. Taken together, the evidence strongly suggests that MMP1-induced cleavage is initiated after Class II pauses and leads to directionally biased motion along the collagen fibril. Furthermore, entry into the gamma distributed Class II pause is temperature dependent and only occurs at periodic locations, indicating that thermally induced local unfolding at susceptible sites may allow MMP1 to initiate collagenolysis.

To further test the collagen degradation model and obtain quantitative details, the authors performed computer simulation of MMP1 trajectories along the collagen fibrils. The results obtained from computer simulation are in close agreement with the experimental data but provide more insights. The authors found that 40%±15% of the pauses at the susceptible sites are Class II pauses and that only 5%±2% of Class II pauses lead to initiation of cleavage. However, once the cleavage is initiated, subsequent cleavages progress rapidly as each cleavage event exposes the next cleavage site. Finally, the simulation suggests that MMP1 preferentially cleaves sites separated by 67 nm on the surface of the collagen fibril.

In summary, this study not only deciphers the molecular mechanism of collagen degradation, but also establishes the single molecule approach as a powerful tool in studying MMP-fibrillar collagen interactions.

Have you heard a good speaker lately?

Please nominate them for our 2013 Retreat, email us at direducation@nhlbi.nih.gov

Recent Publications by NHLBI Fellows

Ahmad, S. M., Tansey, T. R., Busser, B. W., **Nolte, M. T.,** Jeffries, N., Gisselbrecht, S. S., Rusan, N. M., & Michelson, A. M. (2012). Two Forkhead Transcription Factors Regulate the Division of Cardiac Progenitor Cells by a Polo-Dependent Pathway. *Dev. Cell 23*, 97-111

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Park, Y. N., **Morales, D**., **Rubinson, E. H**., Masison, D., Eisenberg, E., & Greene, L. E. (2012). Differences in the Curing of [PSI+] Prion by Various Methods of Hsp104 Inactivation. *Plos One 7*

Rezvani, K., Yong, A. S. M., **Mielke, S**., Savani, B. N., Jafarpour, B., Eniafe, R., **Le, R. Q**., Musse, L., Boss, C., Childs, R., & Barrett, A. J. (2012). Lymphodepletion is permissive to the development of spontaneous T-cell responses to the self-antigen PR1 early after allogeneic stem cell transplantation and in patients with acute myeloid leukemia undergoing WT1 peptide vaccination following chemotherapy. *Cancer Immunology Immunotherapy 61*, 1125-1136

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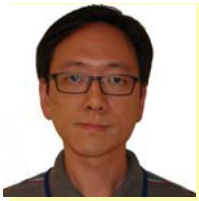
New Fellows

Jie Li, Ph.D., is a Visiting Fellow in the Laboratory of Cardiovascular and Cancer Genetics under Dr. Paul Hwang. Dr. Li earned his Ph.D. in Cancer Biology from Tsinghua University. Previously, he was a Researcher in the Laboratory of Molecular Enzymology at Tsinghua University. Dr. Li's current project is the function of p53 mutation on tumor cell metabolism.

Alex Szatmary, Ph.D., is a Visiting Fellow in the Transgenic Core Facility under Dr. Jian Liu. Dr. Szatmary earned his Ph.D. in Mechanical Engineering at the University of Maryland. Previously, he was a Research Assistant at the University of Maryland Baltimore County campus. Dr. Szatmary's current project is theoretical modeling of how cells generate forces to extend protrusions while crawling.



Jae Wook Lee, Ph.D., is a Visiting Fellow in the Epithelial Systems Biology Laboratory under Dr. Mark Knepper. Dr. Lee earned his Ph.D. in Internal Medicine from Seoul National University College of Medicine. Previously, he was a Researcher in the Nephrology Laboratory at Seoul National University Hospital. Dr. Lee's project at the NIH is deep sequencing (RNA-seq) of renal tubule segment transcriptome.

New Principal Investigator

Nehal Mehta, M.D., is a Principal Investigator in the Cardiovascular Pulmonary Branch. Dr. Mehta received his Medical Degree from The George Washington University Medical School. From 2007-2009 he was a Post-doctoral Research Fellow in Cardiovascular Medicine/Fellow in Genetic Epidemiology at the University of Pennsylvania. Prior to coming to the NIH he was an Adjunct Assistant Professor at the University of Pennsylvania School of Medicine.

Progress, from pg 1 fact that they are here for training.

When there is a clearly indicated career goal, the rest of the report becomes a way to chart your progress towards this career, and this is the second benefit: it allows you and your mentor to evaluate whether your progress is consistent with your goals. The questions that follow ask you to list your achievements and your future plans, which should be specifically designed to develop a c.v. and talents to make you competitive for your career goals. Thus, if your career goal is to be a PI, then a progress report can present achievements and future plans consistent with this: publications, meeting presentations, grant or fellowship applications, etc. For a scientific career in industry, evidence that you are a good collaborator or plans to establish collaborations would be important. If your goal is outside of the lab, then the Progress Report provides an opportunity to identify the specific requirements for entry to that field, and how you are going to get there. In this system, each successive Progress Report should indicate more achievements and fewer training goals. And once all the training goals have been met, this would signal to both you and your mentor that it's time for you to move on.

While the major benefits are to you (and your mentor), the Report is important to the Office of Education. At at individual level, the Office of Education can provide additional advice or mentoring tailored to your particular needs. Thus, if the report demonstrates a goal to be a PI, and there is a solid record of achievement, you'll be contacted to encourage you to apply for a Career Transition Award, either the K22 or the K99. If the report suggests that progress is not optimal, this provides an opportunity for you to discuss the roadblocks to your progress and how to overcome them. The data on career goals also motivates the Office of Education to provide specific programs and speakers tailored to achieving those goals.

The bottom line is that this report, rather than being a chore, provided a valuable opportunity for all involved to improve training , both for the Fellow and for all of DIR. So please treat it seriously!