BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Duane P. Grandgenett	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) GRANDGDP	

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
Mankato State University, Mankato, MN	B.S.	1966	Chemistry
University of Iowa, Iowa City, IA	Ph.D.	1970	Microbiology

A. Personal Statement.

Our laboratory discovered the retrovirus integrase (IN) in avian myeloblastosis virus in 1978. This fundamental discovery provided the foundation for the identification of the HIV IN in 1986. HIV IN plays the critical role along with cellular cofactors in the integration of HIV into DNA of host cells. The pathogenesis of HIV is also influence by selection of host target sites in latently infected cells which results in viral reservoirs.

Our work has centered on the virology, genetics, biochemistry, and biophysics of retrovirus IN. We had developed an efficient physical assay for concerted integration in 1994 using purified AMV IN and large size (>1 kbp) DNA substrates. We attempted to crystallize the recombinant full-length RSV IN (1-286 aa) without and with DNA oligonucleotides here at St. Louis University for several years but were not successful. Currently, in collaboration with Dr. Hediki Arhara (University of Minnesota), we are working on the atomic resolution of RSV IN (1-270 aa IN constructed by our lab) which is fully functional for concerted integration and 3' OH processing. Dr. Arhara has successfully obtained the atomic structure of IN (2.8 Å) and of a two domain fragment (Catalytic core domain/C-terminal domain) at a high resolution of 1.8 Å. We are performing functional analyses of RSV IN mutants to map IN-IN interactions necessary for concerted integration activity. A manuscript is submitted. These structural studies have provided valuable experience for our proposed studies on HIV IN-DNA complexes.

We first published on the recombinant HIV IN in 1991. Merck initiated their collaboration with our lab in 1994 and I held a Merck Investigator Initiated Study Program grant which ended on 12/31/2011. We successfully used purified HIV virions in 1995 to demonstrate that the virion-associated IN was capable of efficient concerted integration, using the above physical integration assay with HIV DNA substrates. Since then, we have published numerous papers using recombinant HIV IN to understand the HIV concerted integration. The lab was first to establish a physiologically relevant IN-to-viral DNA stoichiometry for concerted integration. This result allowed us to determine that a 1:1 stoichiometric relationship existed between IN and LEDGF/p75 for concerted integration. These above results were confirmed and extended by others. The Craigie group used our assay conditions to first identify HIV IN-DNA complexes on native agarose gels. Subsequently, we identified the transient synaptic complex (SC) which is physiologically comparable to the cytoplasmic preintegration complex observed in virus-infected cells. These observations expedited our current studies to understand how Raltegravir and other clinically relevant strand transfer inhibitors inactivate the SC, thus preventing concerted integration. These studies also lead to the identification of a new IN-single DNA (ISD) complex produced only in the presence of certain inhibitors. The ISD complex may be more amenable than the SC for our proposed co-crystallization studies. Further examination of the mechanisms involved in SC assembly lead us to determine that our purified HIV IN existed predominantly as monomers. Our purified HIV IN has a unique property, shown for the first time without the use of a cellular cofactor, to efficiently use viral

DNA oligonucleotides (18 bp to 42 bp) for concerted integration. This work was published last October (Biochem. 2011, 50:9788-9796). Importantly, our observed activity with HIV IN and oligonucleotides is identical to concerted integration activity catalyzed by PFV IN monomers, used for crystallization studies of the PFV intasome. These recent major advancements with HIV IN and our past and current crystallization studies with RSV IN support our attempts to **1**) to determine the functionality of HIV monomers in the assembly of the SC, **2**) to identified and to produce homogeneous HIV IN-DNA complexes without and with strand transfer inhibitors, and **3**) to determine conditions to crystallize IN-DNA complexes using robotic technologies and to resolve the atomic structure of these important and significant nucleoprotein complexes.

B. Positions and Honors

Positions and Employment

1971-1973 Postdoctoral Fellow, Inst. Molec. Virol. (IMV), Saint Louis Univ. Sch. Med. (SLUSM), St. Louis
1973-1977 Assistant Professor, IMV, SLUSM, St. Louis, MO
1977-1981 Associate Professor, IMV, SLUSM, St. Louis, MO
1983-1992 Director, Cell and Molecular Biology Graduate Training Program, SLUSM, St. Louis, MO
1981-present Professor, IMV, SLUSM, St. Louis, MO

Honors.

1971-1973 Postdoctoral Fellowship, American Cancer Society
1977-1982 Faculty Research Award, American Cancer Society
2011 Fellows Award for Outstanding Achievement in Science (Academy of Science St. Louis)
2012 University of Minnesota Distinguish Alumni Achievement Award

NIH Study Sections

1978 Path. Chem.
1981 Exp. Virology Study Sect.(twice)
1983-87 Exp. Virology Study Sect., permanent member, 1988 and 1996 (once each)
1982 Exp. Virol. NCI Cancer Special Program Advisory Committee
1989 NIH Study Section, AIDS and Related Res. Review Group
1997 NIH Special Emphasis Panel: Structural Biology of AIDS Related Proteins
2001-02 Ad Hoc AIDS AARR-1 Study Section (once each)
2004 NIAID PO1 HIV-1 Preclinical/Clinical Program Project
2005 AIDS Drug Discovery and Therapeutics Study Section
2006 AIDS Special Emphasis Scientific Review Group, ZRG1 AARR-A (03)
2007 AIDS Research Review Committee (SRRC)
2007 Special Emphasis Scientific Review Group 2007/10 ZRG1 AARR-D (09) M
2009 AIDS Research Review Committee (SRRC)
2011 AIDS/HIV Drug Development ZRG1 AARR-D 03 M
2012 Council ZRG1 BCMB-H 02, 2012/10

NSF/Other Study Sections.

1978-1988 Ad Hoc Reviewer; NSF
2002 The Wellcome Trust, UK
2006 AIDS Fonds,The Netherlands
2009 Research Council, K.U., Leuven (twice)
2011 Foundation Scientific Research, Belgium
2011 Flanders Research Foundation
2012 Netherlands Organization for Scientific Research (NWO, the Dutch Research Council).

Memberships

American Society for Microbiology, American Society for Virology, The Protein Society, American Society for Biochemistry and Molecular Biology, The Biophysical Society.

Ad Hoc Reviewer (~10 papers per year from listed journals)

J. Virology, Virology, J. of Biological Chemistry, Biochem. Biophys. Acta, EMBO J., Cancer Research, J. of General Virology, Science, Biochemistry, Proc. Natl. Acad. Sci., Nucleic Acid Research, Cell, Genes and Development, Antimicro. Agents and Chemotherapy, PLoS ONE, Retrovirology, J. Clinical Virology, PLoS Pathogens, American J. of Pathology, Biophysical Chemistry.

C. Selected peer-reviewed publications (Selected from 99 total)

Most relevant to the current application

1. Pandey, K.K., Bera, S., Zahm, J., Vora, A., Stillmock, K., Hazuda, D., and **Grandgenett,** D. P. (2007). Inhibition of HIV-1 concerted integration by strand transfer inhibitors which recognize a transient structural intermediate. J. Virol. 81:12189-12199. PMC2169005.
2. Pandey, K.K., Sinha, S., and **Grandgenett,** D. P. (2007). Transcriptional co-activator LEDGF/p75 modulates HIV-1 integrase mediated concerted integration. J. Virol. 81:3969-3979. PMC1866116.
3. Zahm, J., Bera, S., Pandey, K. K., Stillmock, K., Hazuda, D. and **Grandgenett,** D. P. (2008). Mechanisms of human immunodeficiency virus type-1 concerted integration as related to strand transfer inhibition and drug resistance. Antimicrob Agents Chemother. 52:3358-3368. PMC2533447.
4. **Grandgenett**, D. P., Pandey, K. K., Bera, S., and Vora, A. C., Zahm, J., and Sinha, S. (2009). Biochemical and biophysical analyses of concerted (U3/U5) integration. *In* Mechanistic and Pharmacological Analyses of HIV-1 Integration, ed. Engelman, A., Elesvier, Methods 47:229-236. PMC2693883.
5. Bera, S., Pandey, K.K., Vora, A. C., and **Grandgenett,** D.P. (2009) Molecular interactions between HIV-1 integrase and the two viral DNA ends within the synaptic complex that mediates concerted integration. J. Mol. Biol. 389:183-198. PMC2791363.
6. Pandey, K.K., Bera, S., Vora, A. C. and **Grandgenett**, D.P. (2010) Physically Trapping of the HIV-1 synaptic complex by different structural classes of integrase strand transfer inhibitors. Biochem. 49:8376-8387. PMC2965028.
7. **Grandgenett**, D. .P, Korolev, S. (2010) Retrovirus Integrase-DNA structure elucidates concerted integration mechanisms.Viruses. 2:1185-1189. PMC2946245.
8. Bera, S., Pandey, K.K., Vora, A. and **Grandgenett**, D.P. (2011) HIV-1 integrase strand transfer inhibitors stabilize an integrase-single blunt-ended DNA complex. J. Mol. Biol. 410:831-846. PMC3123398.
9. Pandey, K. K., Bera, S., and **Grandgenett**, D. P. (2011) The HIV-1 integrase monomer induces a specific interaction with LTR DNA for concerted integration. Biochem. 50:9788-9796. PMID:21992419. PMC Journal-In progress.
10. Ke, S., Pandey, K. K., Bera, S., Grandgenett, D. P., and Aihara, H. (2012) The asymmetric C-terminal domain dimer of Rous sarcoma virus integrase serves as a viral DNA binding surface. Submitted.

Additional recent publications of importance to the field

1. Sinha, S., Pursley, M., and **Grandgenett,** D.P. (2002). Efficient concerted integration by recombinant human immunodeficiency virus type-1 integrase without cellular or viral cofactors. J. Virol. 76:3105-3113. PMC136053.
2. Makharashvili, N., Koroleva, O., Bera, S., **Grandgenett,** D.P., and Korolev, S. (2004). A novel structure of DNA repair protein RecO from Deinococcus radiodurans. Structure 12:1881-1889.
3. Sinha, S., and **Grandgenett,** D.P. (2005). Recombinant HIV-1 integrase exhibits a capacity for full-site integration in vitro comparable to that of purified preintegration complexes from virus-infected cells. J. Virol., 79:8208-8216. PMC1143728.
4. Bera, S., Vora, A.C., Chiu, R., Heyduk, T., and **Grandgenett,** D. P. (2005). Synaptic complex formation of two retrovirus DNA attachment sites by integrase: A fluorescence energy transfer study. Biochem. 44:15106-15114. PMID16285714
5. **Grandgenett,** D. P. (2005). Symmetrical recognition of cellular DNA target sequences during retroviral integration. Proc. Nat. Acad. Sci. USA, 102:5903-5904. PMC1087955.
6. Pandey, K. K., and **Grandgenett,** D. P.(2008). HIV-1 integrase strand transfer inhibitors:Novel insights into their mechanism of action. Retrovirology:Research and Treatment. 2:41-46. PMC2776739.
7. **Grandgenett,** D. P. (2011). pp32 is Hot. *In* HIV-1 integrase: Mechanism and inhibitor design. *ed.*,

Neamati, N. and Wang, G. Wiley Press, June (Invited Book Chapter).

D. Research Support.

Ongoing Research Support:

NIH 1R21AI100682-01 Grandgenett (PI) (07/01/12-06/30/14)
Title: HIV Integrase Structural Biology
The major goal is to determine why HIV monomers facilitate the assembly of the active tetramer onto viral DNA for concerted integration. We will explore conditions to produce soluble HIV IN-DNA complexes, without and with strand transfer inhibitors, for co-crystallization studies.

Completed Research Support:

NIH 1-R21/R33 AI081629-02 Grandgenett (PI) (06/05/09-05/31/12)
Title: HIV integrase/DNA complexes and concerted integration.
The major goals are to apply biochemical and biophysical approaches to investigate the transient HIV synaptic complex in the concerted integration pathway in vitro.

St. Louis University President's Fund Grandgenett (PI) (03/13/11 to 3/31/12)
Title: The three-dimensional structure of the intact avian retrovirus integrase and its biological significance.
The goal is to study the structure-functional relationships between IN subunits for concerted integration.

Merck Investigator Initiated Grant Grandgenett (PI) (03-16-10 to 12/31/11)
Title: A new major HIV-1 integrase-viral DNA complex produced by clinically relevant strand transfer inhibitors.
The major goals are to investigate a newly identified major IN-viral DNA complex produced in the present of clinically relevant inhibitors.

NIH 2-RO1 CA16312-32 Grandgenett (PI) (05/01/79-04/30/09)
Title: Avian retrovirus DNA synthesis and integration.
The major goals were to study the mechanisms associated with the integration of retroviruses. We had used genetic, biochemical, and biophysical approaches to investigate the IN-IN and IN-viral DNA interactions necessary for concerted integration into host chromosomes. We have now successfully produce crystal structures of fully functional RSV IN in collaboration with Dr. Hideki Aihara (Univ. Minn.).

St. Louis University Seed Grant Grandgenett (PI) (11/01/09-10/31/10)
Title: Identification of a new major HIV-1 integrase-viral DNA complex that contains a strand transfer inhibitor.
The goal was to investigate a new HIV IN-DNA complex produced in the presence of certain strand transfer inhibitors.