BIOGRAPHICAL SKETCH

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NAME: Xinzhong Dong

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

POSITION TITLE: Professor of Neuroscience, Dermatology, Neurosurgery

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
Holy Cross College, Worcester, MA	A.B.	1992	Chemistry
University of California, Los Angeles Thesis Advisor: S. Larry Zipursky	Ph.D.	1998	Cell cycle, neuroscience
California Institute of Technology Advisor: David J. Anderson	Postdoc.	2004	Neuroscience

A. Personal Statement

My laboratory has taken a multidisciplinary approach to understand the cellular and molecular mechanisms of different types of somatosensations including pain and itch, which are initiated and mediated by primary sensory neurons in dorsal root ganglia (DRG). We identified a novel family of G protein-coupled receptors (GPCRs) in mice called Mrgprs. We found that MrgprA3 function as a receptor for chloroquine (an anti-malaria drug) and is required for chloroquine-induced itch. In addition to itch, certain Mrgprs play an inhibitory role in spinal central sensitization and chronic pain. Besides itch receptors, we found that MrgprX2 in humans and MrgprB2 in mice are exclusively expressed in mast cells and play an essential role in IgE-dependent mast cell activation and mediate drug-induced pseudoallergical reactions. In addition, we have generated humanized MrgprX mice in which human MrgprX cDNA is expressed under the promoter of mouse Mrgpr genes. Using the novel mouse line, we are collaborating with various pharmaceutical companies to develop drugs target human Mrgprs. My lab has also generated Pirt-GCaMP mice which allow us to do in vivo DRG imaging. This powerful technique has been used by many labs to reveal novel pain mechanisms. The results from these projects have been published by top peer-reviewed journals. As PI on several private- and NIH-funded grants, I am responsible for overall administration, direction of the project, training several postdoctoral fellows and graduate students, and setting up collaborations within and outside of Johns Hopkins University. Six of my graduate students have graduated and they are currently doing research related works. All of them have published first author papers in high impact journals like *Cell, Neuron, Immunity, PNAS,* etc. Five of my postdoctoral fellows have moved on to take independent faculty positions (four in the US and one in China) and they are all funded by R01s or R01 like grants. My postdocs have been funded with NIH K99/R00, T32, F32 grants, and Damon Runyon Fellowship, etc. I have trained 18 postdocs who have done extremely well and published multiple papers in high impact journals like *Cell, Nature, Neuron, Nature Neuroscience*, *Immunity, Science Signaling, eLife, PNAS*, and *Pain*. In summary, I have developed successful research programs and trained many talented young scientists.

1. Q. Liu, Z. Tang, L. Surdenikova, S. Kim, K.N. Patel, A. Kim, F. Ru, Y. Guan, H. Weng, Y. Geng, B.J. Undem, M. Kollarik, Z.F. Chen, D.J. Anderson, and **X. Dong**. (2009) Sensory neuron-specific GPCRs Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell *139*: 1353-1365. PMCID: PMC2989405
2. A.Y. Kim, Z. Tang, Q. Liu, K.N. Patel, D. Maag, Y. Geng, and **X. Dong**. (2008) Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell *133*: 475-485. PMCID: PMC2605970

3. McNeil B, Pundir P, Meeker S, Han L, Undem B, Kulka M, **Dong X**. Identification of a mast cell specific receptor crucial for pseudo-allergic drug reactions. Nature 2015 *519*:237-241. PMCID: PMC4359082
4. Meixiong J, Anderson M, Limjunyawong N, Sabbagh MF, Hu E, Mack MR, Oetjen LK, Wang F, Kim BS*, **Dong X***. Activation of mast cell-expressed mas-related G-protein-coupled receptors drives non-histaminergic itch. Immunity 2019 *50:* 1163-1171. Preview by Immunity. *co-corresponding authors

B. Positions and Honors

Positions and Employment

09/98-09/04	Postdoctoral fellow, Division of Biology, California Institute of Technology, Pasadena, CA
11/04-03/11	Assistant Professor, Dept. of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD
09/09-08/15	Early Career Scientist, Howard Hughes Medical Institute
04/11-02/15	Associate Professor, Department of Neuroscience, Johns Hopkins University, School of Medicine
02/15-present	Professor, Departments of Neuroscience, Dermatology, Neurosurgery, Johns Hopkins University, School of Medicine,
09/15-present	Investigator, Howard Hughes Medical Institute

Honors

05/92	A.B. Magna Cum Lauda; College Honor at Holy Cross College
06/91-09/91	Fellowship of the New England Consortium for Undergraduate Science Education
09/94-09/97	Graduate student traineeship of National Research Service Award
09/97-09/98	UCLA Dissertation Year Fellowship
12/98-12/00	Postdoctoral fellowship of National Research Service Award
01/01-12/03	Postdoctoral fellowship of American Cancer Society
05/05-04/08	The Whitehall Foundation Fellow
05/05-05/07	Alfred P. Sloan Research Fellow
07/05-06/07	NARSAD Young Investigator Award
07/05-06/06	Klingenstein Fellowship Award
08/2011	Young Investigator Award, Chinese Biological Investigator Society (CBIS)
11/2016	Donlin Long Pain Service Award

C. Contributions to Science

Identification of Itch Receptors

Knowledge of itch receptors other than histamine receptors is largely unknown, despite much of itch being histamine-independent. Our lab delved into this overlooked area of clinical importance and discovered Mrgprs play a critical role in sensing itch. Mrgprs are a family of GPCRs consisting of more than 50 members in the mouse genome. Strikingly, many mouse and human Mrgprs are specifically expressed by small-diameter sensory neurons in the dorsal root ganglion (DRG). Primary sensory neurons in the DRG play an essential role in generating somatosensation by detecting sensory stimuli through their peripheral axons in the skin. To determine the function of Mrgprs *in vivo* while overcoming the potential problem of gene redundancy, we generated a mouse line in which a cluster of 12 Mrgpr genes was deleted (*Mrgpr-cluster*$\Delta^{-/-}$ mice) including MrgprA3 and MrgprC11. The analysis of *Mrgpr-cluster*$\Delta^{-/-}$ mice, together with other evidence, suggests that Mrgprs function as itch receptors that are directly activated by several itch-inducing compounds. For example, the anti-malarial drug chloroquine, which causes unbearable itch in many black Africans, directly activates DRG neurons through MrgprA3 to induce scratching. Bovine adrenal medulla peptide 8–22 (BAM8–22), an endogenous peptide, evokes scratch in mice through its receptor MrgprC11. More importantly, our subsequent human psychophysical study confirmed that skin application of Bam8-22 induces strong itch sensation in every individual tested, validating our mouse studies (Sikand et al., 2011 Journal of Neuroscience). As a result of our findings, many researchers have been using Mrgprs and their itchy agonists to investigate various itch and pain mechanisms. Therefore, the identification of Mrgprs as itch receptors opens new avenues for itch research and the development of novel anti-itch drugs.

a. Q. Liu, Z. Tang, L. Surdenikova, S. Kim, K.N. Patel, A. Kim, F. Ru, Y. Guan, H. Weng, Y. Geng, B.J. Undem, M. Kollarik, Z.F. Chen, D.J. Anderson, and **X. Dong**. (2009) Sensory neuron-specific GPCRs Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell *139*: 1353-1365. *Featured by Cell with a Preview*. PMCID: PMC2989405

b. Q Liu, HJ Weng, KN Patel, Z Tang, H Bai, M Steinhoff, **X Dong**. The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and hyperalgesia. 2011 Science Signal *4*: ra45.
c. L Han, N Limjunyawong, Z Li, OJ Hall, W Mitzner, BJ Undem, BJ Canning, **X Dong**. Mrgprs on vagal sensory neurons contribute to bronchoconstriction and airway hyperresponsiveness. 2018 Nature Neuroscience 21:324-328. PMCID: PMC5857222
d. J Meixiong, C Vasavda, D Green, Q Zheng, L Qi, SG Kwatra, JP Hamilton, SH Snyder, **X Dong**. Identification of a bilirubin receptor that may mediate a component of cholestatic itch. 2019 eLife *8*: e44116.

Identification of itch-sensing neurons

For nearly a century, the somatosensory field has debated whether itch-specific nerve cells exist. Pain-sensing neurons and itch-sensing neurons are known to be alike, with itch often described as pain's little brother. So it is thought that the same set of neurons may conduct two different sensations, pain and itch. Other theories suggest this is not the case, rather the two sensations use completely different sets of neurons, i.e. there is a dedicated population just to conduct itch sensation. My laboratory used elegant genetic techniques to demonstrate, for the first time, that itch-specific nerve cells do exist. We discovered that MrgprA3+ neurons are itch-dedicated neurons that are distinct from pain-sensing neurons. MrgprA3+ neurons are found within DRG where they account for 5% of the total DRG population. We demonstrated that selective activation of MrgprA3+ neurons, even by a normally painful stimulus like capsaicin, leads only to itch sensation. Amazingly, MrgprA3+ nerves exclusively innervate the skin, explaining why we do not feel itch from internal organs. The genetically marked MrgprA3+ neuron mouse line generated from this study has been widely requested by researchers and is an invaluable tool with which to investigate itch mechanisms. For instance, in a recent collaborative study we found that under contact dermatitis conditions, MrgprA3+ neurons spontaneously fired and exhibited hyperexcitability. This finding highlights the importance of these neurons in chronic itch conditions. Therefore, this study has revolutionized the itch research field, resolving long-standing mysteries and debates and has opened the door to novel effective therapies for chronic itch.

a. Han L, Ma C, Liu Q, Weng H, Cui Y, Tang Z, Kim Y, Nie H, Qu L, Patel K, Li Z, McNeil B, He S, Guan Y, Xiao B, LaMotte R, and **Dong X**. A subpopulation of nociceptors specifically linked to itch. (2013) Nature Neuroscience *16*: 174-182. Covered by The New York Times, The Baltimore Sun, Huffington Post, Scientific American Mind, Doctor Radio, NBCnews.com, and other foreign news agencies, etc. PMCID: PMC3557753
b. S Sun, Q Xu, C Guo, Y Guan, Q Liu, **X Dong**. (2017) Leaky gate model: intensity-dependent coding of pain and itch in the spinal cord. Neuron *93*: 840-853. PMCID: PMC5324710

Identification of Pirt and Pirt2: regulators of TRP channels

The family of transient receptor potential (TRP) channels has attracted great interest due to its importance in various sensory systems including thermosensation and nociception. Six TRP channels have been reported to sense different temperature ranges in mammals, including four heat-activated channels (TRPV1-4) and two cold-activated channels (TRPM8 and TRPA1). These channels are also activated by chemical compounds such as capsaicin, mustard oil, and menthol for TRPV1, TRPA1, and TRPM8, respectively. TRP channel activities can be modulated by various mechanisms, including phosphorylation of intracellular residues, increased membrane expression, and association with other proteins. In addition, phosphatidylinositol-4,5-bisphosphate (PIP_2) has emerged as a major regulator of many TRP channels including TRPV1, TRPA1, and TRPM8. We previously showed that Phosphoinositide interacting regulator of TRP (Pirt), a two-transmembrane domain protein that binds to PIP_2, also modulates TRP channels, enhancing the sensitivity of TRPV1 and modulating its role in heat pain and itch. A later study suggests Pirt is an endogenous regulator of TRPM8. In addition, we identified another structurally related protein, called Pirt2 (a.k.a Tmem100). Pirt2 exhibits a different binding specificity towards TRPs: it binds TRPA1 and TRPV1 but not TRPM8. We have shown that Pirt2 positively regulates TRPA1-mediated currents and pain conditions in a TRPV1-dependent manner. More importantly, a small cell penetrating peptide derived from the Pirt2 C-terminus strongly inhibits chronic pain. Pirt and Pirt2 define a novel family of TRP channel regulators. Our functional analyses should facilitate general understanding of TRP modulation and thereby open the door for the development of novel analgesics.

a. A.Y. Kim, Z. Tang, Q. Liu, K.N. Patel, D. Maag, Y. Geng, and **X. Dong**. (2008) Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell *133*: 475-485. PMCID: PMC2605970
b. Patel K, Liu Q, Meeker S, Undem BJ, **Dong X**. Pirt, a TRPV1 modulator, is required for histamine-

dependent and independent itch. PLoS ONE 2011: *6*: e20559. PMCID: PMC3105090

c. Tang Z, Kim A, Masuch T, Park K, Weng H, Wetzel C, **Dong X**. Pirt functions as an endogenous regulator of TRPM8. Nature Communications *4*, 2013. PMCID: PMC3748931

d. Weng HJ, Patel K, Jeske NA, Bierbower SM, Zou W, Tiwari V, Zheng Q, Tang Z, Mo G, Wang Y, Geng Y, Zhang J, Guan Y, Akopian A*, **Dong X***. Tmem100 is a regulator of TRPA1-TRPV1 complex and contributes to persistent pain. Neuron 2015 *85:* 833-846. *corresponding authors. *Previewed by Neuron.* PMC4336228

MrgprX1/C11 inhibits persistent

Besides itch, we found that MrgprX1/MrgprC11 plays an inhibitory role in persistent pain by blocking voltage activated calcium channels and neurotransmitter releases from central terminals of primary sensory neurons in the spinal cord. In addition, we have generated humanized MrgprX1 mice in which human MrgprX1 cDNA is expressed under the promoter of mouse MrgprC11 in the DRG. Using the novel mouse line, we characterized agonists and positive allosteric modulators (PAM) of MrgprX1 to inhibit persistent pain in mice.

a. Guan Y, Liu Q, Tang Z, Raja SN, Anderson DJ, **Dong X**. Mas-related G-protein-coupled receptors inhibit pathological pain in mice. PNAS 2010: *107*: 15933-8. PMC2936626

b. He SQ, Li Z, Chu YX, Han L, Xu Q, Yang F, Liu Q, Tang Z, Wang Y, Hin N, Tsukamoto T, Slusher B, Wei F, Raja SN, **Dong X***, Guan Y*. MrgC agonism at central terminals of primary sensory neurons inhibits neuropathic pain. Pain 2014 *155*: 534-544. MPC3945061 *corresponding authors

c. Li Z, Tseng PY, Tiwari V, Xu Q, Wang Y, Han L, Wu Z, Cui Y, He SQ, Tiwari V, Sun S, Zheng Q, Cheng Y, Huang J, Geng Y, Xiao B, Peng J, Hopkins CR, Raja SN, Guan Y, **Dong X**. Targeting human Mas-related G-protein-coupled receptor X1 to inhibit persistent pain. PNAS (2017) 114 E1996-E2005 PMC5347560.

Functional imaging of DRG neurons

Pain-sensing neurons in dorsal root ganglion (DRG) and trigeminal ganglion (TG) play an essential role in pain transmission and modulation. While the cell bodies reside in the DRG and TG, the axons of these primary sensory neurons innervate peripheral tissues where they detect painful stimuli. Pain signals are transmitted from the periphery to their central axonal terminals in the spinal cord's dorsal horn, where they synapse onto secondary neurons. It is recognized that tissue injury induces hyperexcitability or sensitization of primary afferent neurons, a major contributor to chronic pain. By specifically expressing a genetically-encoded Ca^{2+} sensitive indicator in almost all DRG and TG neurons in Pirt-GCaMP3 mice, we successfully imaged for the first time primary sensory fiber activity at the levels of the skin and dorsal horn. More importantly, we were able to detect robust hyperexcitability in the injured and uninjured nerves. We demonstrated, for the first time, that TRPV1, a major pain sensor in the peripheral nerves, plays an essential role in the sensitization of the central terminals by promoting excitability via a descending serotonin (5-HT) facilitation. In addition, using Pirt-GCaMP3 mice with in vivo DRG imaging, we demonstrated that gap junctions in the DRG contribute to injury-induced neuronal coupling and persistent pain. Pirt-GCaMP3 mice provide the sensory biology field with a unique tool to visualize primary sensory neuron activity under physiological and pathological conditions and to reveal peripheral sensitization.

a. Kim YS, Chu Y, Han L, Li Z, LaVinka PC, Caterina M, Dubner R, Wei F, and **Dong X**. Central terminal sensitization of TRPV1 by descending 5-HT facilitation modulates chronic pain. Neuron 2014 *81*: 873-887. PMCID: PMC3943838

b. YS Kim, M Anderson, KS Park, Q Zheng, A. Agarwal, C Gong, Saijilafu, L Young, SQ He, PC LaVinka, F Zhou, D. Bergles, M. Hanani, Y Guan, DC Spray, **X Dong**. Coupled activation of primary sensory neurons contributes to chronic pain. Neuron 2016 *91:* 1085-1096. PMCID: PMC5017920

Identification of mast cell specific receptor

Mast cells play significant roles in various biological processes and diseases by secreting histamine and many inflammatory and immunomodulatory substances. While classically they are activated by IgE antibodies, a unique property of mast cells is their antibody-independent responsiveness to a range of cationic substances, collectively called basic secretagogues, including inflammatory peptides and drugs associated with allergic-type reactions. We found that basic secretagogues activate mouse mast cells in vitro and in vivo through a single receptor, MrgprB2, the orthologue of MrgprX2. We demonstrated that MrgprB2 and MrgprX2 are targets of many small molecule drugs associated with systemic pseudo-allergic, or anaphylactoid, reactions and anaphylactoid reactions,. More recently, we found that MrgprB2/X2 are a receptor for

proinflammatory peptide Substance P and mediate neurogenic inflammatory pain. These discoveries introduce a mouse model to study mast cell activation by basic secretagogues and identify MrgprX2 as a potential therapeutic target to treat various disease conditions. In addition, we identified MrgprB2/X2 is a receptor for bacterial quorum sensing molecule peptides and elicits anti-bacterial effects.

a. McNeil B, Pundir P, Meeker S, Han L, Undem B, Kulka M, **Dong X**. Identification of a mast cell specific receptor crucial for pseudo-allergic drug reactions. Nature 2015 *519*:237-241. PMCID: PMC4359082
b. Green DP, Limjunyawong N, Gour N, Pundir P, **Dong X**. A mast cell specific receptor mediates neurogenic inflammation and pain. Neuron 2019 *101*: 412-420. Preview by Neuron. PMID: 30686732;
c. Meixiong J, Anderson M, Limjunyawong N, Sabbagh MF, Hu E, Mack MR, Oetjen LK, Wang F, Kim BS*, **Dong X***. Activation of mast cell-expressed mas-related G-protein-coupled receptors drives non-histaminergic itch. Immunity 2019 *50:* 1163-1171. Preview by Immunity. *co-corresponding authors
d. Pundir, P., Liu, R., Vasavda, C., Serhan, N., Limjunyawong, N., Yee, R., , Zhan, Y., Dong, X.,Wu, X., Zhang, Y., Snyder, S., Gaudenzio N., Vidal, J., **Dong, X**., Identification of mammalian receptors for quorum sensing molecules and their role in antibacterial immunity. Cell Host & Microbe 2019 *26:*114-122 Preview by Cell Host & Microbe and Science Signaling

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/xinzhong.dong.1/bibliography/40828532/public/?sort=date&direction=ascending

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

Investigator, (Dong, X.) 09/01/15 – 08/31/21 1.4 calendar months
Howard Hughes Medical Institute
Innate immunity
Major Goals: To investigate cellular and molecular mechanisms of innate immunity.
Role: P.I.

RF1 NS113883 (Dong) 7/01/19 – 6/30/24 1.8 calendar months
NIH/NINDS
Sympathetic-mediated sensory neuron cluster firing as a novel therapeutic target for neuropathic pain
Major Goals: Study the contribution of sympathetic innervation in the DRG to nerve injury-induced spontaneous pain
Role: Multi-PI

R01NS054791 (Dong) 7/15/16-4/30/21 2.4 calendar months
NIH/NINDS
Functional Analysis of Mrgpr Family in itch sensation
The major goals of this project are to determine the role of Mrgpr positive neurons in itch and how Mrgprs genes mediate chronic itch.
Role: PI

R01AI135186 9/01/18 – 8/31/22 2.4 calendar months
NIH/NIAID
Characterization of a dendritic cell specific receptor critical for SJS
Major Goals: To study the role of Mrgprs in Stevens Johnson Syndrome
Role: PI

University of Nebraska (Dong, Sub-PI) 9/15/17 – 2/29/20 0.6 calendar months
NIH/NIDA
Optimization of MrgX1 allosteric agonists as potential therapies for chronic pain
Major Goals: Testing MrgX1 allosteric agonists on HEK cells expressing MrgX1
Role: Sub-PI