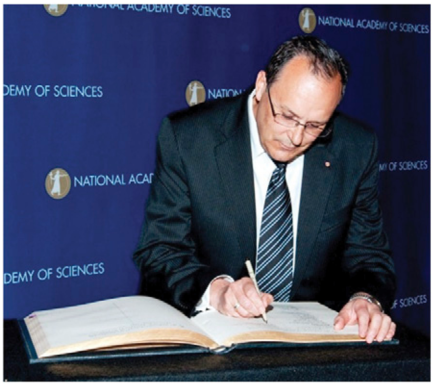
PNAS PNAS PNAS PNAS

Profile of Alexander S. Raikhel

Mosquitoes rely on blood for nutrition, putting them in position to transmit some of the world's deadliest diseases, like malaria and Dengue fever. Alexander S. Raikhel, elected to the National Academy of Sciences in 2009, believes that preventing the mosquito from carrying the pathogen in the first place is the key to vector control. Raikhel has been studying the connection between blood meals and egg production in *Aedes aegypti* in hopes of co-opting egg production signals to activate the mosquito's immune system against incoming pathogens. In his Inaugural Article, Raikhel, a distinguished professor at the University of California, Riverside continues an investigative strategy that has served him well throughout his career: using the latest research in established model organisms to hammer out methods for the mosquito, this time looking for microRNAs active in the egg production cycle (1).



Alexander S. Raikhel.

Tropic of Siberia

Growing up in Siberia, Raikhel's exposure to science outside the former Soviet Union came through the printed page. "My development was through reading books," he said, recalling how he had felt drawn through the pages as if he had accompanied Darwin on the voyages of the HMS Beagle. By age 8 years, he says, "I was really keen on being a scientist." By the time he entered fourth grade, he knew he would go to university to study biology. His parents were both in the medical field, his father a surgeon and his mother a surgical nurse, and Raikhel's father encouraged his son's interest in biology if only, Raikhel says, because he did not want him to pursue his other area of interest: art. In addition to his early convictions, Raikhel had strong notions about what scientists looked like, to the extent that he begged his parents for eyeglasses and faked his eye examination to get them. "I wanted glasses so badly to be a scientist."

In 1965, Raikhel applied to St. Petersburg (formerly Leningrad) State University, one of Russia's oldest and most highly respected schools. However, being Jewish meant that quotas existed for admission, and he was not accepted. Undeterred, Raikhel took evening courses and worked in the Leningrad Zoo as part of the cleaning crew—a hard awakening for a teenager not accustomed to harsh life. Despite these odds, he emerged from the university's extended program in 1970 with a master's degree in zoology and parasitology, having investigated the reproduction and developmental stages of the trematode in an effort to interrupt the worm's lifecycle and prevent disease (2). Raikhel intended to go directly into a doctoral program, but again, he met quota obstacles and was forced to wait several years. During that time, Raikhel worked as a technician in the laboratory of renowned acarologist Yu. S. Balashov at the Zoological Institute of the Russian Academy of Sciences. Finally, in 1973, Raikhel matriculated, choosing to stay in Balashov's laboratory for his thesis and study another parasite: this time, the tick *Hyalomma asiaticum*. He defended his doctorate degree in 1975, having published 11 papers and two book chapters from his thesis research.

While using electron microscopy to study the tick's development during the often week-long feed that swelled the parasite to the size of an acorn, Raikhel says, "I developed a fascination for blood-feeding organisms" (3–5). Siberia is not exactly a nexus of tropical parasitic diseases, but he recalls, "I was traveling in my imagination to the tropical areas, far away from cold Siberia, to appreciate the importance of research on parasitic organisms, causing much human suffering." Soon, he and his wife, whom he met at the University, were looking for a warmer climate to raise their children. However, leaving proved daunting. "We had no idea what was behind the iron curtain," he explained. "Not having much exposure to the larger scientific community, we were untested in our scientific credentials," he says. Raikhel recounts that University of Georgia professor Jerry Paulin had taken a sabbatical at the Institute of Cytology in St. Petersburg, where Raikhel's wife Natasha worked, and later provided the couple assistance in securing positions at his home institution in the United States.

Lifetime Relationship

Arriving in Athens, Georgia, in 1979, Raikhel began a postdoctoral research position with Arden O. Lea, who studied mosquitoes. "He was famous for intricate microsurgery," Raikhel says, recalling Lea's ability to remove brain cells from the tiny insect. "This was where I switched to mosquitoes," he says, a move that would drive vector biology research throughout his professional career. Soon, Raikhel realized that his unique training had prepared him to approach the research with a different perspective, one that he has nurtured and maintained even after 30 years in the United States. "I trained myself to look at biological dynamics," he says. Raikhel credits Michael Locke, a remarkable insect cell biologist, whose publications influenced his early development as a scientist and encouraged Raikhel to consider the fascinating changes in organisms as they develop from larvae to adults. Finally, secure in the knowledge that his training behind the Iron Curtain had engendered a different way of viewing current questions in his field, he says, "I opened a new book and started to investigate."

When Raikhel began to characterize mosquito proteins and receptors important in egg production, such as a yolk protein precursor called vitellogenin, it was the early 1980s. Although the application of biochemical and molecular biological techniques for mosquitoes proved extremely challenging, Raikhel has never been one to give up easily. He again used electron microscopy to achieve his goals and later acquired National Institutes of Health funding to use biochemical methods of investigating vitellogenin biosynthesis. The work localized vitellogenin to an organ in the mosquito called the fat body, which metabolizes lipids in a manner reminiscent of the mammalian liver. Raikhel began to probe deeper into this secretory protein by adapting monoclonal antibody technology (6, 7). He found himself off to what he calls "quite a successful beginning" with *Ae. aegypti*, the primary mosquito carrier of Dengue fever.

Raikhel has continued to use *Ae. aegypti* in his research. "We call it the white rat of vector biology," Raikhel says, citing the ease of the experimental model's use. *Ae. aegypti* is native to the desert, and the eggs can be stored for at least 1 year without much fuss, he explains. "You can store them like seeds of *Arabidopsis*," bringing some of the convenience of plant biology's amenable model organism to a less-developed system. At any given time, there are thousands of mosquitoes housed in containers in Raikhel's lab, taking their meals from rats, chicks, or mice.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 22391.

In 1986, Raikhel joined Michigan State University's Department of Entomology as an associate professor. There, he continued to identify and characterize the ligands and receptors important for egg production in the mosquito. "We did a lot of biochemistry, which was quite thorough." Having investigated the structure and synthesis of vitellogenin, Raikhel wanted to know how the protein travels from the fat body to the oocyte, where it eventually gets broken down into protein for the larva (8, 9). The work included isolating and cloning the first insect vitellogenin receptor (VgR) without any modern genomics tools (10, 11). "Biochemical isolation and cloning were very laborious at the time." To start, there was the question of raw materials. "For each receptor binding reaction, we needed 2,000 mosquito ovaries," he says, explaining that they became proficient at harvesting the minute structures, barely the size of a pinhead. "We had a factory." They found that VgR belongs to the family of low-density lipoproteins (LDL) best known for the cholesterol-carrying human LDL receptor (11). That pioneering research opened the door for exploration of receptors involved in egg maturation in insects, says Raikhel.

Transmission and Control

Mosquitoes acquire and transmit parasites during blood meals, and Raikhel says that the role of feeding in development may hold important clues for determining how transmission might be prevented. Two hormones—juvenile hormone and ecdysone—control egg production and in turn, synthesis of vitellogenin in mosquitoes. Looking upstream, Raikhel wanted to decipher how blood meals translate, molecularly speaking, into egg development. Vitellogenin production peaks at 24 hours postblood meal and shuts off 6–8 hours later (12). Studying how ecdysone initiates this cascade over the course of his career, Raikhel has used ever-evolving biological techniques: cell biology to biochemical methods followed by the tools of molecular biology and now, genomics and bioinformatics. "In the pregenomic era, it was trial and error, cloning of every element of the pathway," he recalls. "It was work from scratch."

Raikhel's lab cloned the mosquito ecdysone receptor (EcR) in 1995 and characterized it as a heterodimer of two nuclear receptors (13, 14); he went on to perform binding studies and probes to understand how nuclear receptors and transcription factors regulate ecdysone's action and thus, egg production (15). In the case of ecdysone, the signals that stimulate vitellogenin synthesis at the proper place and time are related to those that control metamorphosis in *Drosophila* (16). Not content with broad brushstrokes, Raikhel wanted to know exactly which transcription factors binding in what order initiate the egg production cascade after a blood meal (17–20). "Deciphering developmental switches governing the cyclicity of egg production in mosquitoes is essential, because these cycles of blood feeding and egg production serve as a basis for pathogen transmission." Having achieved significant progress (21, 22), Raikhel's lab continues to tackle this driving question.

Discerning which transcription factors are active at certain times in the egg production cascade provided a window for Raikhel to begin practical studies of vector control. The location of egg production precursors in the fat body underscored his conviction that boosting the mosquito's own immune system can prevent transmission. Because the fat body links directly to the mosquito's hemolymph, the equivalent of human blood, the organ can effectively deliver immune boosters straight to where they are most effective. In 2000, Raikhel introduced a genetically modified mosquito with altered immunity (23). Using the vitellogenin promoter to drive the immunity genes, Raikhel could overexpress or knockdown certain immune pathways in hopes of developing a mosquito with an immune system robust enough to kill any ingested parasites, thus preventing transmission. Indeed, Raikhel's group recently developed a transgenic mosquito incapable of pathogen transmission because of the upregulation of two antimicrobial peptides: defensin and cecropin (24). In addition to producing mosquitoes resistant to disease, the work led to transgenic models and other tools—a major breakthrough that finally allowed researchers to begin performing more sophisticated work in mosquitoes.

These genetic tools allowed Raikhel to extend his search for immune factors produced by mosquitoes that are potentially harmful to disease pathogens. In the last 10 years, his laboratory has elucidated major immune pathways in *Ae. aegypti* (25–29). More recently, they helped sequence the genomes of two mosquito species, *Ae. aegypti* and *Culex quinquefasciatus*, significant vectors of Dengue fever and West Nile virus, respectively (30, 31), and searched the genomes for immune-related genes and pathways (32, 33).

Going West

Around the same time that Raikhel developed a transgenic mosquito, he visited the University of California, Riverside to give a talk. On that March day, he recalls, "the blooming fruit trees and beautiful weather" stood in stark contrast to Michigan's winter. Less than 2 years later, Raikhel migrated from the cold Midwest to join the University of California Riverside faculty in the Department of Entomology, later starting the university's Center for Disease–Vector Research in 2005. Raikhel has continued to dissect the connection between the ingestion of blood and egg production. His previous work had elucidated in detail how the hormone ecdysone acts as the major regulator of *vg* expression, but activation of the gene seemed to be conditional: without prior blood feeding, ecdysone does not stimulate egg maturation in mosquitoes. Raikhel and his group discovered that, to jump start egg development, blood-feeding mosquitoes like *Ae. aegypti* rely on an evolutionarily conserved mode of nutritional signaling known as the target of Rapamycin (TOR) signal transduction pathway. Raikhel explains that the pathway, which is ubiquitously expressed in eukaryotes, mediates nutritional signals in other organisms to play key roles in cell growth, proliferation, and cancer. Mosquitoes, however, use amino acid-based TOR signaling in a unique way, adapting it for their lifestyle as blood-feeding insects (34–36).

In an attempt to uncover yet another layer in the complex regulatory network that links blood feeding and egg development in mosquitoes, Raikhel and his group investigated the role of microRNAs (miRNAs)—short RNA sequences that help control translation by preventing the translation of certain coding RNAs. "I became fascinated with these, because so many miRNAs regulated developmental events in *Drosophila* or plants," he says. In his Inaugural Article, Raikhel looks for miRNAs that play key roles in mosquito reproduction (1). Previous studies had explored the potential roles of miRNAs in reproduction but not enough to satisfy Raikhel. He says, "We asked the questions through the eyes of vector biologists."

Raikhel and his group began by examining the expression patterns of 27 miRNAs known to be active in the fat body. One in particular, miR-275, stood out for its sevenfold increase in expression after a blood meal. Further characterization of miR-275 showed that it was activated by the same pathways that activate vitellogenin, TOR, and ecdysone. Bart Bryant, a postdoctoral fellow in Raikhel's laboratory, developed an antagomir—a reverse cRNA sequence—to block miR-275 in vivo. Without miR-275, the female mosquitoes could not digest a blood meal, and large boluses lodged in their guts. Furthermore, the ovaries of the same females failed to develop, revealing another link between feeding and egg production (1). Although Raikhel acknowledges that other important miRNAs may exist, he suggests that miR-275 is among the biggest regulators of reproductive events in *Ae. aegypti.* Because miR-275 is conserved among other arthropod disease vectors, he hopes that it might have a universal and therefore, targetable role in transmission. For now, the team is looking for targets of miR-275 in the mosquito, a task that is more difficult than it would be in a model organism. "I am a freshman in this field," he says, but if his track record shows any indication, it will not slow him down.

Raikhel's determination to manipulate the intractable mosquito using techniques developed for less complicated species has earned him accolades in the field, including the 2001 Entomological Society of America Award in Insect Physiology, Biochemistry, and Toxicology and a 2002 National Institutes of Health MERIT Award; additionally, he was a 2009 Fellow of the Entomological Society of America. He attributes his success in large measure to a loving and supportive family. Being married to another extremely busy academic meant that the couple shared all responsibilities of raising their two sons, Eugene and Vincent, with both parents intimately involved in day to day activities of the kids. Juggling parenting with an intense academic career, says Raikhel, proved challenging for him but very rewarding. "I am blessed by having two wonderful sons. Raising them is one of my major achievements in life," Raikhel says. Even at home, his love for research follows him. He has a passion for French cooking and credits his success in the kitchen to his flair for science. "It's like in a lab. You experiment, and you follow recipes." However, in Raikhel's case, it is the creativity to adjust the recipe and a determination to cook up new ways of studying a bloodsucking pest that makes all of the difference.

Tinsley H. Davis, *Freelance Science Writer*

1. Bryant B, MacDonald W, Raikhel AS (2010) microRNA *miR-275* is indispensable for blood digestion and egg development in the mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA*107:22391–22398.
2. Dobrovolskij AS, Raikhel AS (1973) Life cycle of Haplometra cylindracea Zeder 1800 (Trematoda, Plagiorchiidae). (In Russian: Translation No. RTS 10612, British Library, London). *Vestnik Leningradskii Unversitet Seriia Biologii* 28:5–13.
3. Raikhel AS (1975) An electron microscopical study of intracellular digestion in the midgut cells of the tick *Ityalomma osiaticum:* Formation of primary and secondary lysosomes. *Tsitologia* 17:748–753.
4. Balashov YS, Raikhel AS (1978) Ultrastructure of the midgut in *Ornithodoros papillipes* (Acarina, Argasidae) nymphs in the blood assimilation period. *Parazitologiia* 12:21–26.
5. Raikhel AS (1983) Digestive system. *An Atlas of Ixodid Tick Ultrastructure*, eds Raikhel AS, Hoogstraal H (Entomology Society of America, College Park, MD), pp 59–97.
6. Raikhel AS, Lea AO (1983) Previtellogenic development and vitellogenin synthesis in the fat body of a mosquito: An ultrastructural and immunocytochemical study. *Tissue Cell* 15:281–299.
7. Raikhel AS (1987) Monoclonal antibodies as probes for processing yolk protein in the mosquito; a high-resolution immunolocalization of secretory and accumulative pathways. *Tissue Cell* 19:515–529.
8. Dhadialla TS, Raikhel AS (1990) Biosynthesis of mosquito vitellogenin. *J Biol Chem* 265:9924–9933.
9. Cho WL, Deitsch KW, Raikhel AS (1991) An extraovarian protein accumulated in mosquito oocytes is a carboxypeptidase activated in embryos. *Proc Natl Acad Sci USA* 88:10821–10824.
10. Sappington TW, Hays AR, Raikhel AS (1995) Mosquito vitellogenin receptor: Purification, developmental and biochemical characterization. *Insect Biochem Mol Biol* 25:807–817.
11. Sappington TW, Kokoza VA, Cho WL, Raikhel AS (1996) Molecular characterization of the mosquito vitellogenin receptor reveals unexpected high homology to the Drosophila yolk protein receptor. *Proc Natl Acad Sci USA* 93:8934–8939.
12. Raikhel AS (1992) Vitellogenesis in mosquitoes. *Adv Dis Vector Res* 9:1–39.
13. Cho WL, Kapitskaya MZ, Raikhel AS (1995) Mosquito ecdysteroid receptor: Analysis of the cDNA and expression during vitellogenesis. *Insect Biochem Mol Biol* 25:19–27.
14. Kapitskaya MZ, Wang S, Cress DE, Dhadialla TS, Raikhel AS (1996) The mosquito *ultraspiracle* homologue, a partner of ecdysteroid receptor heterodimer: Cloning and characterization of isoforms expressed during vitellogenesis. *Mol Cell Endocrinol* 121:119–132.
15. Wang S, Miura K, Miksicek RJ, Segraves WA, Raikhel AS (1998) DNA-binding properties of mosquito ecdysteroid receptor/Ultraspiracle heterodimers. *J Biol Chem* 273:27531–27540.
16. Li C, et al. (2000) Conserved molecular mechanism for the stage specificity of the mosquito vitellogenic response to ecdysone. *Dev Biol* 224:96–110.
17. Zhu J, Chen L, Raikhel AS (2003) Post-transcriptional control of the competence factor bFtz-F1 by juvenile hormone in the mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA* 100:13334–13343.
18. Zhu J, Chen L, Sun G, Raikhel AS (2006) The competence factor β Ftz-F1 potentiates ecdysone receptor activity via recruiting a p160/SRC coactivator. *Mol Cell Biol* 26:9402–9412.
19. Sun G, Zhu J, Chen L, Raikhel AS (2005) Synergistic action of E74B and ecdysteroid receptor in activating a 20-hydroxyecdysone effector gene. *Proc Natl Acad Sci USA* 102:15506–15511.
20. Zhu J, Chen L, Raikhel AS (2007) Distinct roles of Broad isoforms in regulation of the 20-hydroxyecdysone effector gene, Vitellogenin, in the mosquito Aedes aegypti. *Mol Cell Endocrinol* 267:97–105.
21. Zhu JS, Miura K, Chen L, Raikhel AS (2000) AHR38, a homolog of NGFI-B, inhibits formation of the functional ecdysteroid receptor in the mosquito Aedes aegypti. *EMBO J* 19:253–262.
22. Zhu JS, Miura K, Chen L, Raikhel AS (2003) Cyclicity of mosquito vitellogenic ecdysteroid-mediated signaling is modulated by alternative dimerization of the RXR homologue Ultraspiracle. *Proc Natl Acad Sci USA* 100: 544–549.
23. Kokoza VA, et al. (2000) Engineering blood meal-activated systemic immunity in the yellow fever mosquito, *Aedes aegypti*. *Proc Natl Acad Sci USA* 97: 1624–1629.
24. Kokoza V, et al. (2010) Blocking of *Plasmodium* transmission by cooperative action of Cecropin A and Defensin A in transgenic *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci USA* 107:8111–8116.
25. Shin SW, Kokoza V, Ahmed A, Raikhel AS (2002) Characterization of three alternatively spliced isoforms of the Rel/NF-κ B transcription factor Relish from the mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA* 99:9978–9983.
26. Shin SW, Kokoza V, Lobkov I, Raikhel AS (2003) Relish-mediated immune deficiency in the transgenic mosquito Aedes aegypti. *Proc Natl Acad Sci USA* 100: 2616–2621.
27. Bian G, Shin SW, Cheon HM, Kokoza V, Raikhel AS (2005) Transgenic alteration of Toll immune pathway in the female mosquito *Aedes aegypti*. *Proc Natl Acad Sci USA* 102:13568–13573.
28. Zou Z, et al. (2008) Mosquito RUNX4 in the immune regulation of PPO gene expression and its effect on avian malaria parasite infection. *Proc Natl Acad Sci USA* 105:18454–18459.
29. Zou Z, Shin SW, Alvarez KS, Kokoza V, Raikhel AS (2010) Distinct melanization pathways in the mosquito Aedes aegypti. *Immunity* 32:41–53.
30. Nene V, et al. (2007) Genome sequence of *Aedes aegypti*, a major arbovirus vector. *Science* 316:1718–1723.
31. Arensburger P, et al. (2010) Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. *Science* 330:86–88.
32. Waterhouse RM, et al. (2007) Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science* 316:1738–1743.
33. Bartholomay LC, et al. (2010) Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens. *Science* 330: 88–90.
34. Hansen IA, Attardo GM, Park J-H, Peng Q, Raikhel AS (2004) Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proc Natl Acad Sci USA* 101:10626–10631.
35. Hansen IA, Attardo GM, Roy SG, Raikhel AS (2005) Target of rapamycin-dependent activation of S6 kinase is a central step in the transduction of nutritional signals during egg development in a mosquito. *J Biol Chem* 280:20565–20572.
36. Park JH, Attardo GM, Hansen IA, Raikhel AS (2006) GATA factor translation is the downstream step in the TOR-mediated amino acid activation of vitellogenin gene expression in the anautogenous mosquito, *Aedes aegypti*. *J Biol Chem* 281:11167–11176.