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OBITUARY

In memoriam—Evgueni Ananiev

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Evgueni Vitalievich Ananiev, 60 years old, died January 10, 2008 after a courageous battle with an aggressive brain tumor. His life, which begun in the difficult post-war years in Soviet Union, was a reflection of the tumultuous change in the world and tremendous progress in biology, to which he made important contributions until his last days. He was a rare breed—a true scientist—dedicating his life to the discovery of chromosomal structures and showing little interest in material rewards (Fig. 1).

Evgueni was born on January 13, 1947 in Moscow to a family of modest means. He seemed destined to a life as a semi-skilled mechanic, attending night school and helping his mother. However, since his early childhood, he dreamed to be a biologist. His first taste of science came as a teenager. With the help of his father's physician, he got a job as a technician at the Institute of Molecular Biology, the laboratory of A. Prokofieva-Belgovskaya, a well-known cytogeneticist and a collaborator of the Nobel laureate Hermann J. Muller. Despite the tragic events of the Lysenko period, which destroyed much of Russian genetics, a few outstanding genetics labs were able to re-build their science in the 1960s, and perhaps the best of them was the scientific community Evgueni was very fortunate to join. There, he learned the craft of cytogenetics and assisted in the study of human chromosomes. This experience had important repercussions for the rest of his life. He was able to enroll in evening classes at Moscow State University and eventually transferred to a day program in Genetics and completed his B.S. in 1970. He later joined the lab of a young assistant professor, Vladimir Gvozdev, a *Drosophila* geneticist at the Institute of Atomic Energy. There, he worked on the old *Drosophila* genetics problems of X-chromosome dosage compensation and position effect of euchromatin translocated to heterochromatin, earning his doctorate in 1975.

The mid 1970s was an exciting time in molecular biology, as cloning was just being developed, and Georgi Georgiev, the prominent Russian biochemist at the Institute of Molecular Biology, was getting into the act. Georgiev was interested in applying these new tools to the understanding of gene structure and his lab succeeded in cloning segments of *Drosophila* DNA. Gvozdev began collaboration with the Georgiev lab to characterize these clones. At that time, Evgueni had a reputation as an experienced cytologist, and he was asked to map the clones onto *Drosophila* polytene chromosomes by *in situ* hybridization. This work led to perhaps the most important discovery of Evgueni's career: While analyzing hybridization pattern of one of the probes (Dm225, later re-named MDG1, a *copia*-like element), Evgueni observed that multiple hybridization signals differed in most of their locations between the two parental chromatids (Fig. 2). He confirmed his observations of polymorphism by analyzing both parental lines. Evgueni boldly hypothesized that the most straightforward explanation for this result was that these "genes" must be moving. Evgueni's colleagues and superiors were not persuaded by his idea, as alternative hypotheses could not be easily excluded (such as ancestral differences between lineages or differential selective amplification). The resulting Science publication (Georgiev et al. 1977) is therefore quite cautious in its interpretation, although "gene migration" is mentioned, with references to Barbara McClintock's work in maize and M.M. Green's work on transposition in

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Fig. 1 Evgueni Vitalievich Ananiev

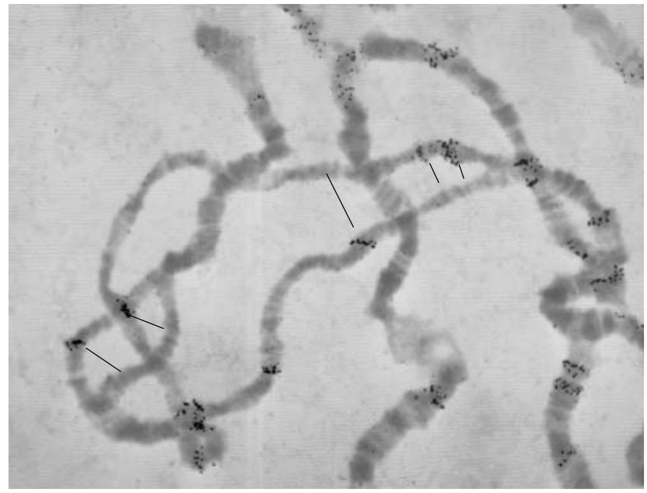

Fig. 2 Reiterated genes with varying location in intercalary heterochromatin regions of *Drosophila melanogaster* polytene chromosomes (Ananiev et al. 1978)

Drosophila.[1] The detailed cytological results were published (Ananiev et al. 1978) but “the jumping gene” hypothesis was not discussed in this paper. Nevertheless, this publication was the first molecular description of transposable elements in eukaryotes. It is worth remembering that understanding of microbial transposons was just developing at the time, and the yeast *Ty* element was yet to be discovered. Evgueni’s hypothesis was confirmed later, as molecular details began emerging, primarily from G. Rubin’s lab. To the end of his life, Evgueni believed that the team he was part of could have received more credit for this discovery had they been more assertive in the interpretation of their results. This work earned Evgueni the highest scientific award, the USSR National Award for Science, and in 1983, he was conferred a Doctor of Science degree. Interestingly, a similar observation of the polymorphisms of retrotransposons was made by DNA sequencing in maize 25 years later (Fu and Dooner 2002; Rafalski and Ananiev 2009).

In 1983, Evgueni was given an opportunity to head the laboratory of plant genetics at the Institute of General Genetics. He initiated work on the barley genome. Several papers were published from his lab regarding the structural organization of B- and C-hordein genes, 5S rRNA genes, the barley-specific repetitive gene family *Dialect*, and of so-called relic DNA, which later turned out to be telomere sequences. Unfortunately, deterioration of the economic situation at the time of “perestroika” made his ambitions for the barley group impossible to achieve. Evgueni and his family immigrated to the United States in 1992, following a path made by many Russian scientists. In the USA, Evgueni was looking for a project that could open new opportunities for him to study chromosomes. This moment came when he saw a position announcement in Science regarding the study of oat–maize chromosome addition lines, developed at the University of Minnesota by the labs of Ron Phillips and Howard Rines. Evgueni sensed a great potential in this novel experimental system, which allowed the isolation of single maize chromosomes in the oat background.

The years 1995–1998, spent at the University of Minnesota, were among the most exciting and productive years in Evgueni’s career. He made fundamental discoveries of the structural elements of the maize centromeres and organization of “knobs”. The oat–maize chromosome addition lines were a unique system for isolation of genes from individual maize chromosomes. Evgueni built cosmid libraries from the oat–maize addition lines and screened them with a mixture of the 20 most abundant maize-specific transposable elements. Using this approach, he isolated a number of cosmids clones from individual maize chromosomes.

Similar approach resulted in the isolation of knobs and centromeres-specific sequences. Knobs are cytologically defined heterochromatic regions of maize chromosomes discovered by B. McClintock as early as 1929. Using a known knob-specific 180-bp repeat as a probe, Evgueni found a novel 350-bp knob-specific repeat. He showed that both 180- and 350-bp repeats form long tandem arrays, which are

[1] We know now that Barbara McClintock observed movement of DNA transposons fundamentally different from the retrotransposons that Georgiev’s group cloned and Evgueni mapped to chromosomes.

hotspots for retrotransposon integration. He found also that some knob clones have a foldback structure similar to the *Drosophila* giant transposons. This provoked a speculation that maize knobs are actually megatransposons.

To attack the problem of understanding centromere structure, Evgeuni searched the literature for any hint of a centromere-specific sequence. A wheat clone identified in Graham Moore's lab was reported in 1996 to hybridize to rice and maize centromeres. Using this sequence as a probe, Evgueni isolated cosmid clones from the oat–maize addition lines containing centromeric sequences of maize chromosome 9. This led to the discovery of two elements specific to the maize centromeres for which he coined the names *CentA*, a retrotransposon, and *CentC*, a short 156-bp satellite-like repeat that appears to be a major component of maize centromeres. His paper (Ananiev et al. 1998) is an important contribution to the molecular understanding of plant centromeres. During this time, he conceptualized making an artificial maize chromosome from these "building blocks"

In 1998, Evgueni joined the Research Division of Pioneer Hi-Bred Intl., a leading maize seed company. Initially, Evgueni's team was involved in physical mapping of the maize genome, but his interests returned to constructing maize artificial chromosomes. The Ananiev team first identified and collected the necessary building blocks, including centromeres, telomeres, selectable markers, and replication origins. During this time, Evgueni's group made fundamental insights into the organization of maize centromeres, most importantly in recognition of the inverted repeat organization of *CentC* sequences at the core of the centromere. This intense team-research effort succeeded in producing maize artificial chromosomes in 2006. Unfortunately, this success was clouded by the diagnosis of glioma shortly afterward. Despite the grim prognosis, Evgueni remained positive and scientifically active as long as his health allowed. His last paper in Chromosoma (14th in this journal) is about to be published (Ananiev et al. 2009) 35 years after his first Chromosoma article (Ananiev and Gvozdev 1974).

I met Evgueni several years ago when his team was involved with the development of the physical map of the maize inbred Mo17. During that time, Evgueni and Mark Chamberlin also made a beautiful microphotograph illustrating amazing polymporphisms in the distribution of repeat families in maize, recapitulating the early *Drosophila* work, but using the latest multi-color digital technology (Rafalski and Ananiev 2009). I continued to visit with him regularly to learn about the progress of the artificial chromosome project. It was clear to me that Evgueni did "science for science's sake" and not for other ulterior motives. His infectious enthusiasm and huge energy always impressed me. When visiting Evgueni and his wife Olga Danilevskaya at their home, I learned that he had many other talents, such as painting in the old master style, photography, and film making and was very accomplished in each of them.

It is tragic that Evgueni Ananiev's life was cut short in the prime of his life. Personally, I am sad that upon arriving at the Pioneer campus in Johnston, I cannot visit his office, see the stunning pictures of maize chromosomes, and hear about his latest ideas about the structural engineering of chromosomes.

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References

Ananiev EV, Gvozdev VA (1974) Changed pattern of transcription and replication in polytene chromosomes of Drosophila melanogaster resulting from eu-heterochromatin rearrangement. Chromosoma 45:173–191

Ananiev E, Gvozdev V, Ilyin YV, Tchurikov N, Georgiev G (1978) Reiterated genes with varying location in intercalary heterochromatin regions of Drosophila melanogaster polytene chromosomes. Chromosoma 70:1–17

Ananiev EV, Phillips RL, Rines HW (1998) Chromosome-specific molecular organization of maize (Zea mays L.) centromeric regions. Proc Natl Acad Sci U S A 95:13073–13078

Ananiev EV, Wu C, Chamberlin MA, Svitashev S, Schwartz C, Gordon-Kamm W, Tingey S (2009) Artificial chromosome formation in maize (Zea mays L.). Chromosoma (in press) doi:10.1007/s00412-008-0191-3

Fu H, Dooner HK (2002) Intraspecific violation of genetic colinearity and its implications in maize. Proc Natl Acad Sci U S A 99:9573–9578

Georgiev GP, Ilyin Y, Ryskov AP, Tchurikov NA, Yenikolopov GN, Gvozdev VA, Ananiev E (1977) Isolation of eukaryotic DNA fragments containing structural genes and the adjacent sequences. Science 195:394–397

Rafalski A, Ananiev E (2009) Genetic diversity, linkage disequilibrium and association mapping. In: Bennetzen JL, Hake SC (eds) Handbook of Maize: History and Practice of Genetics and Genomics. Springer New York (in press)