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Portrait

From Cytometry to Cell Cycle

A Portrait of Zbigniew Darzynkiewicz

Zoya N. Demidenko[1,*]
George P. Studzinski[2]
Mikhail V. Blagosklonny[1]

[1]Department of Medicine; New York Medical College; Valhalla, New York USA

[2]Department of Pathology and Laboratory Medicine; UMD-New Jersey Medical School; Newark, New Jersey USA

*Correspondence to: Zoya N. Demidenko; Brander Cancer Research Institute; 19 Bradhurst Ave.; Suite 2400; Hawthorne, New York USA 10532; Tel.: 914.347.2801; Fax: 914.347.2804; Email: zoya_demidenko@nymc.edu

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As indicated by his somewhat elaborate Slavic name, Zbigniew Darzynkiewicz, a Polish-born American scientist, has very strong ties to Poland. In 1968, he escaped a communist regime and became a role model from foreign lands for a new democratic Poland. Still Polish scientists come regularly to his laboratory in Hawthorne, New York to learn science. The first democratic President of Poland, Lech Walesa, awarded him a gold medal for scientific achievements. He was also awarded the Polish Millennium Gold Award in recognition of research accomplishments which was also bestowed on other luminaries of Polish heritage, including Andrew Schally, Ludwik Gross, Hilary Koprowski and Zbigniew Brzezinski. He was also elected as a foreign member of the Polish Academy of Sciences (Krakow), and just recently, Polish film-makers have produced a movie "Anatomy of Success" that describes some of his achievements.

Dr. Darzynkiewicz has an enormous bibliography. In the current total, he has authored over 450 original publications and over 100 chapters and reviews in books devoted to the subjects of cell growth, regulation of the cell cycle and apoptosis, and has either authored or edited numerous books. His publications have been cited over 20,000 times in the scientific literature. And yet, he still works seven days a week. At 9 a.m., even on Saturdays and Sundays, he is in his laboratory at Brander Cancer Research Institute.

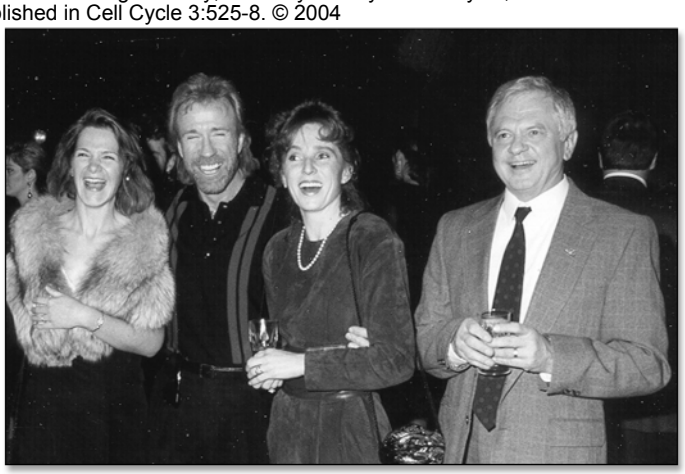
While a high school student, Dr. Darzynkiewicz was fascinated by quantum physics and by cosmology. He was planning to become a physicist. Yet, in communist Poland it was impossible to pursue this dream, so he entered, as if by default, a Medical School. Physics lost, but cell biology won. In his rare spare hours, he still reads books on general relativity, quantum mechanics and cosmology. Not surprisingly, he has introduced many physics-oriented ideas and approaches to cell biology.

Dr. Darzynkiewicz received his M.D. in 1960, and his Ph.D. in 1966 from Warsaw Medical School in Poland. Dr. Darzynkiewicz had escaped from Poland to Sweden as a political refugee in 1968 (just after the invasion of Czechoslovakia by Warsaw pact forces), and was accepted by the laboratory of Nils Ringertz at the Karolinska Institute, Stockholm. He worked there for a year and a half, co-authoring with Nils eight papers. Then, Endre A Balazs offered him the next job and helped his further scientific career in the United States. In 1974, he became associated with the Sloan-Kettering Institute for Cancer Research. Concurrently, he was a Professor of Cell Biology and Genetics at Cornell University Medical School, as well as a Member of the Sloan-Kettering Institute for Cancer Research, the Head of the Experimental Cell Research Laboratory, and the Director of the Flow Cytometry Core Facility Network, all at the Sloan-Kettering Institute, New York. Since 1990, he has been with New York Medical College, Valhalla. Dr. Darzynkiewicz is the Director of the Brander Cancer Research Institute at the New York Medical College and the Professor of Medicine, Pathology and Microbiology.

One of Zbigniew Darzynkiewicz's very important achievements is the creation of Brander Cancer Research Institute. Originally known as the Cancer Research Institute, it officially opened on October 1, 1990 with the arrival of a team of scientists recruited from the Memorial Sloan-Kettering Cancer Center in New York City. Dr. Frank Traganos was appointed as an Associate Director of the Institute. By that time Dr. Traganos had already been working with Dr. Darzynkiewicz for more than 15 years in areas involving carcinogenesis and treatment of cancer. They had co-authored at least 144 papers, and many book chapters.

In 1998 the Cancer Research Institute changed its name to the Brander Cancer Research Institute (BCRI) to honor the late Joel Brander, who unfortunately succumbed to cancer. Joel Brander and his wife Julie Brander established "This Close" Foundation for Cancer Research which supports research at the BCRI.

Zbigniew Darzynkiewicz with owl.



Zbigniew Darzynkiewicz with Chuck Norris. A fund-rising dinner for Brander Cancer Research Institute.

Dr. Darzynkiewicz has been a member of the Editorial Board of *Cell Cycle* from its inception in 2002. He is also the editor or the co-editor of *Cytometry* and *Experimental Cell Research*, and at least 10 other journals including *Leukemia* and the Ukrainian journal *Experimental Oncology*. He is a Past President of The Cell Kinetics Society, and also a Past President of the International Society for Analytical Cytology.

It is difficult to count his publications. While we were starting this article, PubMed retrieved 417 publications but in two weeks the number become 419!

For the last 5 years, PubMed retrieves 102 of his publications. Dr. Darzynkiewicz is one of the most prolific scientists in cell biology. His most recent paper is a seminal review entitled "Cytometry of the cell cycle: Cycling through history".[1] And so we will take you on our own journey, starting from his first publication.

His first article (at least, the first article available on PubMed) was published in 1963 in *Experimental Cell Research*. Between 1965 and 1968, he was the first author of six *Nature* and *Science* papers.[2-5] Among papers published in 1969–1971, there were eight publications co-authored by N.R. Ringertz, with whom he was working at the time. In 1974, we notice the first publication with Dr. Melamed and Dr. Traganos,[6] who became his main collaborators. Together, they succeed in developing cytology automation by flow cytometry.[7]

Dr. Darzynkiewicz's research then concentrated on cell biology with a particular focus on cancer cell growth and the regulatory mechanisms associated with cell growth and progression through the cell cycle. He developed several techniques that have world-wide application with which scientists can analyze metabolic parameters of the cell that are related to cell cycle kinetics, cell sensitivity, anti-tumor drugs and apoptosis. Thereby, Dr. Darzynkiewicz created new fields of science and significantly changed cell biology and cytology.

One major advance was development of multiparameter analysis in individual cells. Initially, Zbigniew and co-authors indicated that several parameters of stimulation of individual lymphocytes could be measured simultaneously by flow-cytofluorometry after differential staining of cellular DNA and RNA with the metachromatic fluorescent dye acridine orange.[8] This methodology had a long way to evolve from DNA and RNA to oncoproteins, tumor suppressors, cyclins to caspases and serpases.[9,10] Most prominent was detection of cyclins in individual cells by flow and laser scanning cytometry.[11,12] This methodological progress allowed hundreds of scientists to further investigate cyclins in different phases of cell cycle and find unexpected phenomena, see for example, a recent commentary.[13] Numerous current publications including methodological[14] or mechanistical[15,16] ones can be traced to the methodological fundamentals developed by Dr. Darzynkiewicz and his collaborators.

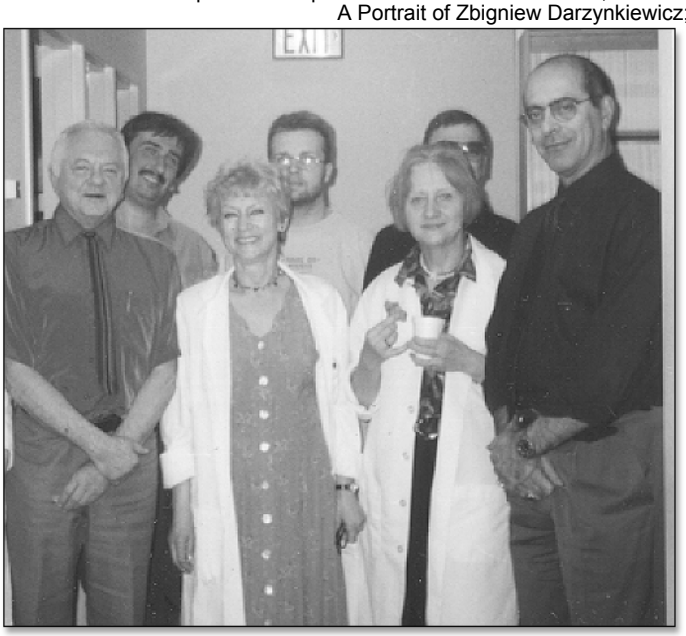
Another breakthrough was the subdivision of the cell cycle into additional sub-phases that cannot be distinguished by regular cytometry, including cytometric distinction of G_0 from G_1 cells,[8] and the recognition of critical restriction points subdividing the G_1 phase of cell cycle as detected by flow cytometry.[17,18]

Initially, flow cytometry could not distinguish G_2 and mitosis (both have 4N DNA content), leading to the term G_2/M. Dr. Darzynkiewicz has devised numerous methods of distinguishing G_2 and M, including such sophisticated procedures as the discrimination of G_2 and mitotic cells by flow cytometry according to different expression of cyclins A and B.[19] Based on these advances, he and collaborators have identified points of growth arrest caused by anti-cancer drugs. For example, staurosporine blocks cell's progression through G_1 between the cyclin D and cyclin E restriction points.[20] Unscheduled expression of cyclin B1 and cyclin E occurs in several leukemic and solid tumor cell lines.[21] As recently reported, overexpression of cyclin E, especially its low molecular weight isoform, can deregulate the cancer cell cycle.[22]

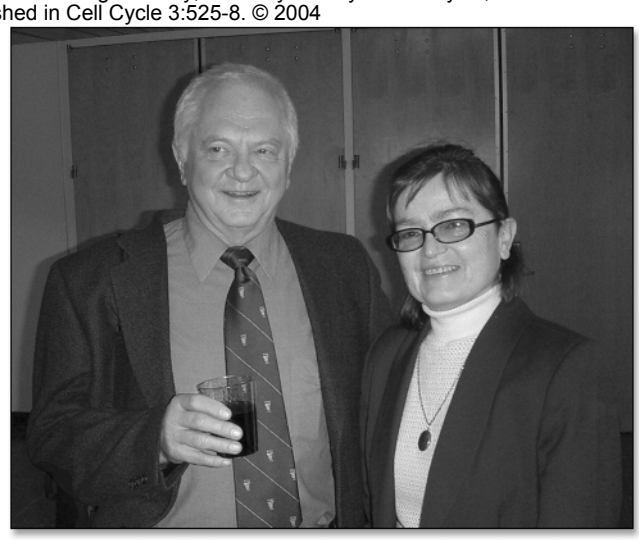
In fact, this knowledge can be especially useful now in designing cell cycle based combinations of anticancer drugs. But more on this later.

The most dramatic scientific input by Dr. Darzynkiewicz is his contribution to the recognition that cell death is as much important as the cell cycle. He has linked conceptually and methodologically apoptosis to the cell cycle. For the first time, cell division and cell death were studied simultaneously. The most cited paper in cytometry (1149 citations) is "Features of apoptotic cells measured by flow cytometry".[23]

Latter he coined the word necrobiology in "Cytometry in cell



At Brander Cancer Research Institute. First row: Z. Darzynkiewicz (Director), Dorota Halicka, Barbara Ardelt, Frank Traganos (Associate Director); second row; P. Smolevski, J. Grabarek, J. Kunicki



Zbigniew and Elizabeth Darzynkiewicz.

necrobiology: analysis of apoptosis and accidental cell death (necrosis)".[24] This paper has been cited more than 440 times.

Dr. Darzynkiewicz also made an important contribution to the study of mechanisms of cell death caused by tumor necrosis factor (TNF). Cells, ordinarily resistant to the cytotoxic action of TNF can be rendered sensitive by treatment with the inhibitor of transcription actinomycin D (AMD). This implies that a combination of TNF and AMD may be considered in oncology for treatment of tumors otherwise unresponsive to TNF alone.[25] This idea was not clinically implemented, in part because AMD is also a DNA damaging drug and cannot be used at high doses to inhibit transcription in patients. In an unexpected twist, TNF can be combined with another inhibitor of transcription, namely flavopiridol.[26] Flavopiridol, which was used as inhibitor of CDKs, turned out to be potent and reversible inhibitor of transcription in clinically relevant concentrations.[26]

The link between cell death and cycling brought about even more significant advances: cell cycle-dependent effect of anticancer drugs, killing of cells in particular phases of the cell cycle. They demonstrated cell cycle-specific effects of topoisomerase inhibitors and lovastatin.[27-30] Furthermore, cycle arrest in G_1, caused by camptothecin (CAM) was likely protective. Exposure of mouse lymphocytic L1210 cells to camptothecin (CAM) causes a slowdown in the rate of cell progression through S and G_2 phases of the cell cycle; the "terminal" point of CAM action is about 1 h prior to mitosis. In contrast, treatment of human promyelocytic HL-60 cells with CAM results in the immediate (occurring as early as 2 h after treatment) death of S- and G_2+M-phase cells. The data indicate that there may be a tissue (leukemia type) specificity in the response of cells to camptothecin and suggest that myelogenous leukemias, especially those characterized by high proliferation rates, may be especially sensitive to the cytotoxic action of this and perhaps other topoisomerase I inhibitors.[31]

Advances in necrobiology were accelerated by development of two methodologies: a method of detection of apoptosis by rapid and automatic identification of DNA strand break[32-34] (this method is currently known as TUNEL assay) and of Laser-scanning cytometry.[35]

Using the enzyme terminal transferase, Dr. Darzynkiewicz and his collaborators developed TUNEL assay and used it to identify the cell cycle position of apoptotic cells. Cells progressing through S phase were selectively susceptible when treated with camptothecin, teniposide, m-AMSA, Mitoxantrone, H7, hydroxyurea, and 1-β-D-arabinofuranosylcytosine. Cells in G_2-M preferentially underwent apoptosis in cultures treated with H7 or with γ-irradiation. Cells in G_1 phase were preferentially affected by 5-azacytidine, nitrogen mustard, and hyperthermia. The cell cycle related difference in susceptibility to apoptosis may be a reflection of both the severity of the lesion induced by a given drug and the ability of the cells to repair that lesion; both can vary depending on the cell cycle phase.[36] The method of detection of DNA strand breaks (3'-hydroxyl termini) in individual cells offers several advantages and can be applied to clinical material (tumor biopsies) to study the induction of apoptosis in tumors during treatment, as a possible prognostic marker. The protein-associated DNA breaks in the "cleavable" DNA-topoisomerase complexes are the primary lesions induced by the inhibitors and precede apoptosis, were not detectable by the present methods.[33]

Fascinating technical advance was the development of a laser-scanning cytometer (LSC), a microscope-based cytofluorometer, which has attributes of both flow and image cytometry.[35]

How can we compare parameters in living cells with those measured only in dead cells? As an admirer of quantum physics, Dr. Darzynkiewicz found an analogy between cytometry and measurements in quantum physics. Like Erwin Schrodinger, he enlisted the assistance of a cat to illustrate how the act of measurement affects reality of the measurement event.[37] As Schrodinger pointed out, until the event is observed the Schrodinger's cat remains in an indefinite state, the alive cat being superimposed on the dead one. Taking advantage of the "file merge" feature of the laser-scanning cytometer, Dr. Darzynkiewicz have been able to correlate the supravital changes that occur during apoptosis, namely the drop in mitochondrial transmembrane potential (Delta Psim) and the generation of the reactive oxygen intermediates (ROIs), with features revealed by analysis of fixed cells: the cell cycle position and DNA fragmentation. The approach has opened a possibility to study direct relationships, within the same cells, between cellular changes (e.g., occurring during apoptosis, mitogenesis, differentiation, etc.) detected by functional assays of live cells and changes that cannot be analyzed supravitally. Therefore, the Schrodinger's cat dilemma in cytometry can be resolved by laser-scanning cytometry (LSC).[37]

Investigation of cycle-dependent cell death and a different response (arrest in G_1 versus arrest in S or no arrest) of normal and cancer cells to growth inhibitors has culminated in very important therapy-oriented notion. First, certain DNA damaging anticancer drugs selectively kill cells in S phase.[27] Second, certain kinase inhibitors arrest normal lymphocytes in G_1 but do not arrest leukemia and cancer cells in G_1, instead allowing them enter S phase.[38] Combining of this two facts lead to the concept of protection of normal cells from chemotherapy.[39,40] Dr. Darzynkiewicz had pointed out that inhibitors of the cell cycle can arrest (in G_0/1 phase) normal cells but not cancer cells. When such inhibitors are administered with the cytotoxic drugs that target proliferating cells, they protect normal cells by sequestering them in the nonsensitive G_0/1-phase compartment, but they offer no such protection to the tumor cells. Intense chemotherapy can then be administered to the cancer patient with less toxic effects.[41] This letter published in JNCI had attracted attention of one of the authors of this editorial (MVB), who was working on the concept of selective protection of normal cells from paclitaxel by inducing G_2 and G_1 arrest.[42] Moving from different directions, both scientists had independently re-discovered the notion of protection of normal cells, initially suggested by Arthur Pardee based on the restriction point of the cell cycle.[43] Different avenues of investigation led to the same conclusion.[44-45]

This chronicle demonstrates the stature of Dr. Darzynkiewicz as a scientist. But he is more than just that. He is a warm, charming, caring person, an excellent educator, a leader in numerous International Workshops, an effective speaker in Invited Lectures and Seminars, and a true friend to many former and current trainees and colleagues. Remarkably for a man of his scientific achievements, he is both a skier and a competitive swimmer of unusual competence, and currently participates in Biathlons that include swimming across a lake in New Jersey Highlands. Thus, Zbigniew Darzynkiewicz is truly a "man for all seasons".

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