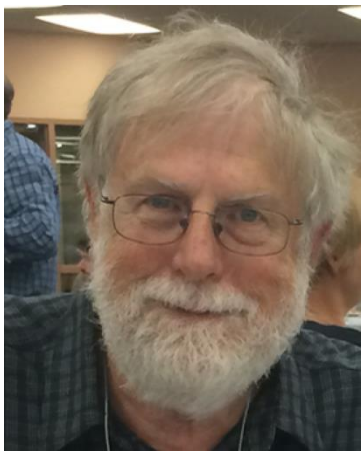
Niels C. Bols

Autobiographical sketch / July 2017



The invitromatics of RTgill-W1: A star of the rainbow trout invitrome with a bright invitroomic future

We recently introduced to in vitro biology three new terms: invitromatics, invitrome, and invitroomics (PMID:28374170). Starting with invitromatics, I would like to illustrate these terms, using as an example the rainbow trout gill epithelial cell line, RTgill-W1 (DOI:10.1111/j.1365-2761.1994.tb00258.x). Invitromatics is the science and history of establishing, characterizing, engineering, storing, and distributing cell lines. Part of invitromatics includes compiling information on cell lines and making this data easily accessible through resources such as the Cellosaurus. The scientists involved might be called invitromaticists or invitromaticians. The history of most cell lines is likely to be anecdotal and could include the biographies of the scientists as well as narratives on development, characterization, storage and distribution of the cell line.

For the history of RTgill-W1, I will begin with myself and end with RTgill-W1 in the American Type Culture collection (ATCC). I was born in Revelstoke BC Canada and raised in Webster's Corners BC. I received my BSc from Simon Fraser University (SFU) and was trained as a cell biologist for my MSc and PhD with respectively Dr Hal Kasinsky at UBC and Dr Art Zimmerman at University of Toronto. I learned about animal cell culturing in the mid-1970s as a postdoc in the laboratory of Dr Nils Ringertz in the Department of Medical Cell Genetics at the Karolinska Institutet, Stockholm, Sweden. In September 1977, I became a faculty member in the Department of Biology at the University of Waterloo (UW) in Ontario Canada and for a few years afterwards continued to work with human fibroblasts. By the early 1980s aquaculture and environmental toxicology were emerging as important disciplines but with little work been done at the cellular level. As a cell biologist, I thought that I might contribute to their progress, plus being in a biology department and working on fish I would have more opportunities to collaborate with colleagues. Rainbow trout were chosen because of their importance to both aquaculture and toxicology. At first my lab used the gonadal cell line, RTG-2, to study at the cellular level the thermal biology of this cold water fish. One of my MSc students at this time was Anngraini Barlian from warm water Indonesia, so when I showed her a flask of RTG-2, I quipped that she was the first person to see rainbow trout cells before she saw a rainbow trout (RT). However I wanted to expand the types of cells that I could use and thought that having an epithelial cell lines from the gill would open many additional research avenues, including environmental toxicology

and studies on fish pathogens. I asked Anggraini as a side project to initiate primary gill cell cultures. Preparing primary cell cultures without any bacterial microbial contamination was difficult, but Anngraini was highly skilled and wonderfully persistent. However, by the time she was consistently successful, she had completed her MSc and had to go back to Bandung. We put her notebooks and the flasks of primary cell cultures in a drawer in my office, which was notoriously cold. About a year later I looked at the flasks and found patches of spread out epithelial cells but also gill fragments. In one flask in particular I noticed sprouts growing out of the gill fragments (see Fig 2B in DOI:10.1111/j.1365-2761.1994.tb00258.x). I speculated that these fragments were primary lamella that had maintained the microenvironment to support stem cell populations and that these stem cells were the source of the sprouts and might lead to a continuous cell line. I subcultivated the flask with trypsin and the two new flasks quickly developed monolayers of epithelial cells that could be further subcultivated repeatedly. The cells were named RTgill-W1, with the W standing for Waterloo and the 1 for the first of possibly more RT gill cell lines from Waterloo. This success led to my lab dictum "don't throw any flasks away", at least without carefully looking at them. Pat Goegan was doing his BSc thesis research project with me and helped to cryopreserve RTgill-W1 and start some characterization. This included finding out that the cells had mycoplasma as detected with H33258 staining. Therefore I sought the help of Dr Lucy Lee at the Department of Veterinary Anatomy at University of Saskatchewan and her colleagues, Sarah Caldwell who was an amazing electron microscopist and Manuel Chirino-Trejo who knew about mycoplasma. Using commercially available antibiotics against mycoplasma, we were able to clean the cells. We put a lot of effort into describing this but the details were removed from the manuscript at the request of reviewers who thought it took up too much space. I think that one of the keys to our success was to plate the cells in the presence of antibiotics and then as soon as the cells had attached to trypsinize them again and repeat the process. During trypsin passaging, the cells round up and then spread and this reorganization might help dislodge any attached mycoplasma so that they are more vulnerable to the antibiotics and/or will be discarded when the cultures are rinsed. A few years later, Liz Joyce in my lab helped in the submission of RTgill-W1 to ATCC.

The invitrome is the grouping of cell lines around a common theme. The theme is the choice of the researcher so RTgill-W1 belongs to many invitromes. For example, RTgill-W1 belongs to the gill invitrome. Another grouping is based on species. The rainbow trout invitrome began in 1962 with RTG-2 but now includes RTgill-W1 and many other cell lines. RTgill-W1 has been widely used and so perhaps can be considered a star of the rainbow trout invitrome. The use of invitromes is invitroomics. Invitroomics is the application of cell lines to study the cellular and molecular biology of multicellular organisms or to manufacture useful products. The term 'invitroomics' might be most easily employed as an adjective. For example, invitroomic ecotoxicology would be the use of multiple cell lines to study problems in ecotoxicology at the cellular level. A related example would be invitroomic environmental monitoring. Recently a Swiss company, aQuaTox-Solutions, has begun to offer services in

evaluating water quality and the environmental risk of industrial products through the use of rainbow trout cell lines, including RTgill-W1. Hopefully RTgill-W1 has a bright business future.

Cell lines established by Niels Bols and his group

RTgill-W1 (CVCL_6441)	ABCA (CVCL_YB40)	ASHe (CVCL_A5TA)
ASimf20 (CVCL_AZ82)	HEW (CVCL_R907)	PBLE (CVCL_R845)
PHL (CVCL_5863)	RTgutF (CVCL_YI84)	RTgutGC (CVCL_DE13)
RT-milt5 (CVCL_AZ89)	RT-ovf1 (CVCL_AZ90)	RTHDF (CVCL_S147)
RTL-W1 (CVCL_L011)	RTP-91E (CVCL_L013)	RTP-91F (CVCL_L014)
RTS11 (CVCL_F835)	RTS34 (CVCL_L015)	RTS34st (CVCL_L016)
WE-afin8e (CVCL_AZ93)	WE-brain3 (CVCL_AZ94)	WE-brain5 (CVCL_AZ95)
WE-cfin11e (CVCL_AZ96)	WE-cfin11f (CVCL_AZ97)	WE-heart1 (CVCL_AZ98)
WE-heart6 (CVCL_AZ99)	WE-liver3 (CVCL_BA00)	WEBA (CVCL_BA03)
WErpe (CVCL_BA04)	ZEB2J (CVCL_6E10)	ZSSJ (CVCL_6E22)