RECOLLECTIONS OF DEMETRIOS A SPANDIDOS

Glasgow, Scotland. November 1986

In the spring of 1976 I was awarded a postdoctoral fellowship from the Medical Research Council (MRC) of Canada to work in the laboratory of Professor Louis Siminovitch in the Department of Medical Genetics at the University of Toronto. I took up the position on 1st June 1976.

My project was to transfer genetic markers (as we called them at that time) or genes (as we call them now) from one somatic cell to the other *in vitro* using the appropriate selection system. The time was right since several such markers in mammalian somatic cells were available and the various selection systems developed. These experiments were based on earlier observations by Szybalska and Szybalski (Szybalska, E.H. and Szybalski, W. Genetics of human cell lines. IV. DNA mediated heritable transformation of a biochemical trait. Proc. Natl. Acad. Sci. USA 48, 2026-2034, 1962) and by McBride and Ozer, (McBride, O.W. and Ozer, H.L. Transfer of genetic information by purified metaphase chromosomes. Proc. Natl. Acad. Sci. USA 70, 1258-1262, 1973) on the transfer of the hprt marker using DNA or chromosomes respectively.

So, right away I began to apply the procedure of McBride and Ozer in order to transfer the markers for methotrexate resistance and amanitin resistance. I thought that the chances of success would be higher using chromosomes rather than naked DNA. This was proved later to be true.

Within a matter of weeks I found out that the chromosomes made by this method were not sufficiently intact and the efficiency of transfer very poor. As it stands today (13 years later) the method has not been of any practical use.

In order to make progress I thought I should try to solve two problems: (1) To improve the quality of isolated chromosomes, and (2) to improve the efficiency of gene transfer. I did an extensive search in the literature and among a number of available methods for isolating chromosomes I selected three that I thought worth trying. After several trials I settled for the procedure of Wray and Stubblefield (Wray, W. and Stubblefield, E. A new method for the rapid isolation of chromosomes, mitotic apparatus, or nuclei from mammalian fibroblasts at near neutral pH. Exp. Cell Res. 59, 469-478, 1970). Of all the methods tried this one gave the purest and most intact chromosomes of Chinese hamster cells that could be seen in the light microscope. Although the method sounds straightforward in the paper it did not prove to be so at the lab bench. I had to play around with conditions quite a bit before I got it right. By that time there was a visitor in the lab - a professor from USA whose last name escapes my memory now, his first name was Marvin. He was working in an unrelated field and wished to jump into somatic cell genetics. He was an open, very friendly and very bright guy with a lot of interests beside science. He soon realised that my experiments were very encouraging and he wanted to jump in. I helped him but he also wanted to try some of his own ideas. Unfortunately, none of his ideas worked in practice. Toward the end of the month he decided to follow my protocol for the isolation of chromosomes. In July I had to go to Cold Spring Harbor Laboratory for a course. Marvin had asked me to call collect after a few days to make sure that everything he was trying was OK, which I did giving him additional advice. When I came back he was proud of his