# Redefining differentiation: Reshaping our ends

**Helen M. Blau**

"There's a divinity that shapes our ends; rough hew them how we will," wrote Shakespeare in *Hamlet*. But is anatomy indeed destiny? As an assistant professor at Stanford University in the 1980s, and after years of growing cells in culture and staring at them under the microscope, I could not believe that once cells differentiated, their fate was sealed forever. Why was differentiation 'one way'? I still remember my excitement when our heterokaryon experiments, in which human differentiated cells were fused with mouse muscle myotubes, demonstrated that the 'terminally' differentiated state of human cells could be altered. The revelation of cell fate plasticity was thrilling. If understood mechanistically, this plasticity might be enlisted for new medical applications. That was the turning point in my career.

Perhaps this insight was facilitated by my own evolution as a person in response to changes in my environment. Aged nine, I lived with an Austrian family, where I learned German, milked cows, experienced the rich taste of freshly laid eggs, and rode Lipizzaner horses. At fourteen, I lived in France, climbing trees to pick fresh figs, cutting grapes in a vineyard, descending the farmer's well in a bucket, eating live sea urchins with a swipe of a baguette, and learning French. As an undergraduate student in England, I became a devotee of tea and crumpets, rubbing brasses in remote Yorkshire churches, and singing Irish ballads in pubs. My London relatives were delighted to note that I had developed an accent indistinguishable from theirs. Clearly, these diverse locales changed me: my tastes, behaviour and language. So why shouldn't the environment of a cell do the same?

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When I started my research career, I wanted to know if cell fate could be changed, and if silent genes typical of other cell types could be activated by altering the cytoplasmic environment 'housing' the nucleus. I also wanted to find out if groups of eukaryotic genes were activated in concert when a threshold concentration of regulators induced a molecular switch in a gene expression program, as was shown in prokaryotic operons by Jacob and Monod in France, as well as by Ptashne in the USA. The power of cell fusion in addressing these questions was clear to me from experiments performed in the 1960s by Harris, Ephrussi, Weiss and Ringertz. These studies provided compelling evidence for the existence of trans-acting repressors of genes; however, they could not determine the existence of activators in the fusion products studied, because they underwent cell division. Mitosis caused the disparate nuclei to combine into one, and chromosomes to be lost and rearranged, so that novel gene expression could well have resulted from loss of a repressor.

I reasoned that I needed an experimental system in which cell division did not occur and the balance of regulators could be altered in a controlled way. Heterokaryons seemed ideal. The nuclei of diverse cell types remained separate in these short-term fusion products, which persisted for several days without dividing. Heterokaryons had been used without success in the 1960s, but the target cell for gene activation in those experiments was the chick erythrocyte, a cell type so specialized that in mammals it even lacks a nucleus. In the 1980s, I decided that this approach warranted revisiting with different cell types. In my third year as an assistant professor, I realized that non-dividing multinucleate muscle myotubes provided a natural framework for stably housing nuclei from different cell types. Moreover, I reasoned that studying whether cell fates could be altered would be facilitated by fusing mouse and human cells, to allow the gene products of each nucleus to be distinguished based on species differences. Aided by able students (referred to by colleagues as 'Helen's Angels'), I realized my dream. I was elated to find that heterokaryons, formed by fusing mouse muscle cells with human amniotic cells, activated previously silent human muscle genes. We extended these findings to all three major cell lineages — mesoderm, ectoderm and endoderm — as represented by human hepatocytes, keratinocytes and fibroblasts, respectively. In mammals, liver or skin does not normally beget muscle, but our experiments revealed that these cells were fully capable of radical change. The relative dosage of cytoplasmic regulators surrounding the nuclei, which resulted from the ratio of the fused cells, determined in which nucleus genes were silenced or activated, with one differentiation program dominating. Our results showed that the stable state of gene expression typical of a differentiated cell is largely governed by 'cytoplasmic factors', which we now know are transcription factors working continuously to maintain that state, and which can reawaken dormant genes to switch on different genetic programs.

Recently, it has been tremendously gratifying to see our heterokaryon work from the 1980s extended to novel approaches in mammalian nuclear reprogramming, such as induced pluripotent stem cells, through the overexpression of four transcription factors. These approaches underscore the fundamental role of the balance of regulators in dictating changes in differentiated somatic cell fate. Now, my dream is to translate these findings into clinical applications in regenerative medicine: divining the means to reshape our ends.

**COMPETING FINANCIAL INTERESTS**
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