TRANSCRIPTION

The Transcriptional Paradox: Octamer Factors and B and T Cells

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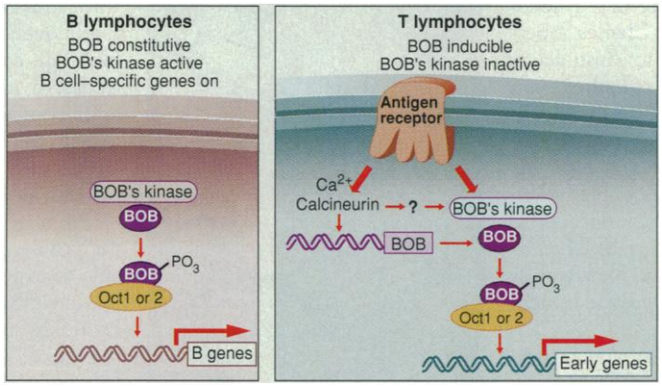
Capturing the moment when a cell becomes committed to a particular identity has been an elusive goal. This pursuit of an understanding of cell specification has followed a trail from DNA to protein. In most cases the trail rapidly arrives at a paradox: Transcription factors are almost never expressed as specifically as their target genes. The latest development in this quest, reported by Wirth and his colleagues on page 221 of this issue (*1*), concerns the Oct transcription factors, which control the cell type–specific expression of immunoglobulins.

The octamer sequence came to fame when transcriptional enhancers were first discovered (*2*–*4*). This short sequence contributes to the B cell–specific enhancer that controls immunoglobulin gene expression, a function first shown in transfection studies and later in intact mice (*5*, *6*). Initially the octamer was thought to control B cell–specific production of immunoglobulin by simply binding the B cell–specific octamer binding protein Oct2. However, a number of observations quickly appeared indicating that B cell specificity was more complex than first assumed (*7*). First, either the ubiquitous Oct1 or the B cell–restricted Oct2 was equally capable of conferring B cell–specific transcription on a reporter gene controlled by a DNA sequence that could bind either Oct1 or Oct2. Second, Oct2 expression did not clearly correlate with immunoglobulin gene expression. Third, Oct2 expressed in nonlymphoid tissues could not activate promoters whose transcription was controlled by an octamer sequence. What appeared to be critical for octamer specificity was the source of the extract used to carry out in vitro transcription or the cell type into which genes were transfected (*8*). These observations recalled earlier studies with the HNF-1 homeodomain protein in which a specific 11-kD cofactor, DCoH, was shown to modulate its activity (*9*). In addition, the activity of Oct1 can be redirected to viral genes by VP16, the herpes virus cofactor for Oct1 (*10*). Thus, the search for B cell specificity was directed to accessory molecules that bind Oct1 or Oct2.

By reconstituting transcription in vitro, the Roeder group discovered an accessory protein they termed OCA-B (also called OBF; however, it can be safely assumed that the Roeder group will probably not object to the name used in the present study, BOB). The gene encoding this factor was novel with no homology to DCoH or VP16. Subsequently, two other groups also isolated BOB (*11*, *12*), and all three groups found that BOB was expressed exclusively in B cells; expression in the thymus and small intestine was interpreted as being due to B cell contamination (*13*). At this point the most fertile ground in the search for B cell specificity appeared to be the factors that controlled BOB.



Different jobs for BOB in B and T lymphocytes. In B lymphocytes (left), BOB is expressed constitutively and BOB's kinase is active, so B cell genes with BOB's target, the octamer sequence, are expressed constitutively. In T lymphocytes (right), BOB is expressed only when activated by agents that mimic antigen receptor signaling. BOB's kinase also becomes active after stimulation, phosphorylating BOB on Ser^{184} and causing transcription of octomer-containing early genes.

Now Zwilling *et al.* report that BOB is induced in T lymphocytes activated either with pharmacologic agents or through their antigen receptor, a result that reemphasizes the question of how the octamer sequence confers B cell–specific expression to its linked genes. The induction of BOB in T cells is inhibited by FK506 and cyclosporin A. This observation provides nearly conclusive evidence that calcineurin is involved because of the exquisite specificity that these agents have for the calcineurin active site (*11*–*13*). Messenger RNA for BOB first appears about 2 hours after stimulation and requires prior gene activation.

But what is BOB doing in T cells that don't ever activate their immunoglobulin genes? The authors suggest that it contributes to the activity of octamer binding sites important for the transcription of the T cell growth factor interleukin-2 (IL-2), IL-5, and other genes. When Oct1 binds to the octamer sequence in the IL-2 gene, it is accompanied by another inducible protein, first termed OAP (*17*), which contained peptides from Jun (*18*). One wonders, however, if the sticky and highly abundant AP-1 complex (consisting of the transcription factors Fos and Jun) may have been purified when BOB was really the activity being sought.

Zwilling *et al.* go on to show that the simple presence of BOB is not sufficient for activation of transcription in T cells, but that another activity must be present. This activity turns out to be a kinase that phosphorylates the activation domain of BOB on Ser^{184}. Mutation of this serine to alanine results in 5 to 10 times less activity.

These new results indicate that the activity of Oct1 is differentially controlled in T cells and B cells (see the figure). Several important questions are raised. First, why are there no T cell defects in the BOB-deficient mice? In the absence of BOB, mice showed defects in late B cell development indicating, but not demonstrating, that BOB is required for normal B cell development. However, no defects in T cell development or activation were noted by any of the three groups that ablated the BOB gene despite the fact that BOB was expressed in activated T cells, at least in one of the studies (*19*). Does the usual response to this result—that BOB is somehow redundant in T cells—apply here? Another, more remote possibility is that BOB falls under the control of the general T cell activation response which, based on RNA hybridization analysis includes over 1000 genes. Could the BOB gene simply be within a chromosomal locus that is activated?

Second, what is the kinase that determines the activating potential of BOB? The Wirth group now has a perfect assay for BOB's kinase and should be able to work backward through this signaling pathway, an approach that has been highly effective for

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the JAK-STAT, mitogen-activated protein (MAP) kinase, and Ca^{2+}-calcineurin pathways. Finally, if BOB and its forthcoming kinase are all that there is to the B cell specificity of immunoglobulin production, then why are the immunoglobulin genes not expressed in activated T cells? We are left with an exciting advance but have confronted the well-known paradox: Transcription factors and the signaling pathways that control them are seldom—maybe never—as specifically expressed as the genes they control. One is tempted to suggest that we don't yet know all the players, or even to revert to that last refuge of scoundrels—chromatin.

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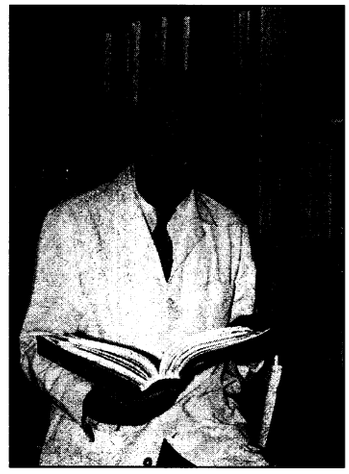
RETROSPECTIVE

John C. Eccles (1903–1997)

Roger A. Nicoll

On 2 May 1997, the neuroscience community lost one of its most influential leaders. Eccles is best known for his work in which he demonstrated that synapses in the brain release chemicals that can excite or inhibit the postsynaptic cell. For this work, he shared the 1963 Nobel Prize with Alan Hodgkin and Andrew Huxley.



Eccles graduated from Melbourne University and attended Oxford University as a Rhodes Scholar, studying under Sir Charles Sherrington. In 1937, he returned to Australia as head of the Kanamatsu Institute of Pathology in Sydney. He moved to New Zealand in 1944 as Professor of Physiology at the University of Otago, and then in 1951 came back to Australia to the National University in Canberra. In 1966, as the mandatory retirement age approached, he moved to the United States, first to Chicago and then to the State University of New York at Buffalo. In 1975 he retired to Switzerland where he lived until his death.

Although best known for his work in the early 1950s on excitatory and inhibitory synapses, for decades before and after this period he dominated the field of neuroscience, touching virtually every aspect of the field. Acetylcholine and Renshaw cells, GABA and presynaptic inhibition, trophic influence of motor nerves on muscle, initial segment and action potential initiation, dendritic action potentials, kinetics of transmitter diffusion in the synaptic cleft, synaptic plasticity, basket cells and inhibition, inhibitory rebound and thalamic oscillations, physiological characterization of cerebellar cortical circuits—these are just a few of his contributions. Each one opened up entire disciplines that are still actively investigated.

But Eccles's most important contribution was his ability to reduce complex problems to simple and exciting concepts. Although his hunger for data was insatiable, he was always after the general principle. This gift is well documented in two of his books, "*The Physiology of Nerve Cells*" and "*The Physiology of Synapses*." These books, which I discovered during my first year in medical school, opened up an entirely new and fascinating world. The message was quite simple: Although the brain is undoubtedly the most complex machine imaginable, one could begin to understand it by studying the individual building blocks, that is, neurons. The neuroanatomist Cajal had also fervently maintained this view throughout his life, but what was so provocative about Eccles's books was that he described how one could, with the aid of microelectrodes, eavesdrop on the ongoing private synaptic communication in a single cell buried deep in the brain. It was now possible to take the beautiful, but static, cellular architecture of Cajal and bring it to life.

From 1973 to his retirement in 1975, I had the privilege of working with Eccles and was able to experience firsthand this larger-than-life character with his childlike curiosity, boundless energy, and extreme tenacity. He, at the age of 72, participated in every experiment (surgery and recording), each of which typically lasted late into the night. The speed and precision with which Eccles performed a spinal laminectomy were breathtaking. I have never encountered anyone so completely consumed by neuroscience, and to this day I and many others remain under his spell. Consequently, it is frustrating that so much of what Eccles contributed seems to be taken for granted. But perhaps this is the way it should be: The really important contributions quickly become second nature to us.

Two of Eccles's most distinguishing traits—his need to understand results in their broadest possible context and his tenacity—were very much in evidence during his retirement. He wrote extensively on the mind-brain problem, vehemently rejecting the materialist view of the mind. Regardless of one's own view on this topic, one had to admire the relentlessness with which he pursued his ideas. As with the scientific period of his life he was a tireless warrior. It is important that his philosophical views, which have few advocates among neuroscientists, not diminish the impact that his extraordinary contributions have had in shaping our understanding of the brain.

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