Developmental biology using purified genes

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Some history
After three years of college I entered the University of Chicago Medical School in the fall of 1952 and discovered biochemistry and research. Lloyd Kozloff, a member of the bacteriophage group in the Department of Biochemistry, guided my research. While in medical school I began searching for a future research subject, thinking it should be an important medically related problem but unexplored by what were then the modern methods of biochemistry.

The field of embryology, newly named ‘developmental biology’, caught my attention. Reproductive biology was barely discussed, and descriptive embryology was taught in two lectures as a part of gross anatomy. In 1953, I attended a biochemistry journal club discussion of the Watson-Crick Nature paper describing the structure of DNA. I knew immediately that my future would involve some merger of DNA research, biochemistry and the field of embryology.

After an internship at Charity Hospital in New Orleans and my wedding, my wife and I moved to Bethesda, Maryland. My two years of service were fulfilled as a member of the first class of ‘research associates’ at the US National Institutes of Health (NIH). I spent two wonderful years in the Institute of Mental Health as part of a project organized by Seymour Kety to investigate systematically the metabolism of molecules that had been implicated in schizophrenia. I was assigned to study the urinary metabolites of the amino acid histidine because it was the precursor of one such compound—histamine. I ended my two years by feeding 10 microCuries of $^{14}$C-histidine to ten healthy controls and ten people with schizophrenia, collecting their urine and analyzing the radioactive products. Then I published one of the few negative studies on the subject of the biochemical basis of schizophrenia.

From the NIH I went to the Pasteur Institute in Paris to study bacterial gene regulation in 1959, the year after the Lac repressor had been discovered. Before leaving Bethesda, by the greatest luck I learned about a small research institution in Baltimore that was associated at that time with the Johns Hopkins Medical School called the Department of Embryology of the Carnegie Institution of Washington. I contacted Jim Ebert, the director, and arranged an advanced postdoctoral fellowship after my year in Paris. It is hard to imagine two more diverse research institutions.

The Pasteur Institute was at the forefront of biology, involved in the founding of molecular biology. Every day at lunch Jacques Monod, François Jacob and André Lwoff presided over an exciting discussion usually augmented by a prominent visitor. The Department of Embryology was just phasing out 50 years of describing the anatomical development of the human embryo. The faculty was principally engaged in experimental embryology. I was the first biochemist. This is how I came to spend 50 years pursuing the topic of gene regulation in development at the Carnegie Institution’s Department of Embryology in Baltimore. I cannot imagine a more ideal place.

The isolation of genes before the recombinant DNA era
I choose embryos of the frog Rana pipiens to study because the eggs could be fertilized in vitro and the embryos developed synchronously and provided unlimited amounts of material for biochemistry. While I was at the Pasteur Institute, two separate groups discovered messenger RNA (mRNA), and it had become clear from studies with bacteria that ribosomal RNA (rRNA) and transfer RNAs were direct gene products. Previously, ‘differential gene action’ had been studied in embryos as fluctuations in enzymatic activity that was assumed to represent changes in the amount of a protein. I realized that, as RNA was closer to the gene than protein was, its analysis would be a more reliable reflection of gene expression changes.

By the early 1960s, sucrose-gradient centrifugation had been devised to separate by size three general classes of RNA, the 18S and 28S rRNA, low molecular weight RNAs and a very heterogeneous DNA-like RNA that was presumed to include the newly discovered mRNA. I found that the three size classes of RNA were synthesized at vastly different rates during development. Even though the unfertilized frog egg is a single cell, it has as many ribosomes as the same weight of a multicellular somatic tissue such as the liver. New rRNA was not synthesized until the embryo had developed to 10,000 cells, and new ribosomes did not contribute substantially to the total content of ribosomes until several days later.

My first discovery at Carnegie was that frog embryos do not develop in distilled water. They need four cations—calcium, sodium, potassium and magnesium, in that order during development. Magnesium deficiency was especially interesting because, without it, embryos develop for several days and arrest as early swimming tadpoles at the same stage that a frog embryo...
begins to increase its total amount of rRNA. It was well known that magnesium holds ribosomes together. In 1962, I read an article about a mutant of the South African clawed toad Xenopus laevis that had an altered number of nucleoli. The wild type had two nucleoli in each nucleus, the heterozygote had one nucleolus and was perfectly normal. Crossing two heterozygotes yielded a typical Mendelian ratio of offspring in which one-quarter of the progeny were anucleolate. These homozygous mutants arrested development exactly at the stage of a typical amphibian oocyte, because they were anucleolate. These homozygous mutants arrested development exactly at the stage of a normal embryo deprived of magnesium. This suggested to me that the mutation affected the synthesis of new ribosomes. The only source of this mutant was the Oxford lab where it had been discovered. Meanwhile, I learned that Xenopus laevis was imported from South Africa for use as a pregnancy test by an entrepreneur who lived just ten miles away in suburban Baltimore. I quickly switched from R. pipiens to X. laevis. John Gurdon at Oxford and I teamed up to show that the anucleolate mutant synthesized no 18S or 28S rRNA. These mutant embryos synthesized no 18S or 28S rRNAs in amphibian oocyte nuclei. We learned that the genes encoding the 5S rRNA genes are not linked to the rRNA genes but comprise an even greater fraction of the genome of X. laevis DNA than does the rRNA. We purified and studied the 5S rRNA genes (5S DNA) from the X. laevis genomic DNA in 1971 (ref 7), taking advantage of the unusual base composition of the many tandemly repeated 5S rRNA genes. These small genes were especially useful for detailed characterization and functional studies in the days before DNA sequencing and recombinant DNA methods were available. We learned that the transcription of the 5S rRNA genes (Fig. 3) in different species of Xenopus was a more complex process than in mammals. The spacers were very different, whereas the genes were highly conserved. Yet the spacers within a species were remarkably similar, a phenomenon that we called horizontal evolution. Within each 5S rRNA gene repeat there is a pseudogene. The first eukaryotic gene to be completely sequenced was one repeat of genomic 5S DNA by Nina Fedoroff and George Brownlee. We learned that there are multiple families of 5S rRNA genes in the X. laevis genome. The largest one is expressed only in oocytes. We called this the ‘dual 5S rRNA gene system’, a developmental problem that we pursued for a decade. 5S DNA is a template for accurate transcription in crude extracts of oocyte nuclei, providing a simple assay for the cis-acting DNA sequences required for accurate initiation and termination of transcription. Once genes could be cloned, we made successive deletions around the gene transcribed properly even when we had deleted one-third of either the 5’ or the 3’ ends of the gene. We called this the ‘internal control region’. Bob Roeder and his colleagues purified the first eukaryotic transcription factor from X. laevis oocytes. This protein, called TFIIIA, binds the internal control region of the 5S rRNA gene. The discovery of gene amplification in 1968 encouraged us to ask whether this mechanism is used more broadly when a cell synthesizes a large amount of a gene product. I had read about the unusual amino acid sequence and the huge size of the protein silk fibroin from the silkworm Bombyx mori. Most of the protein consists of repeats of a single gene. I was encouraged to ask whether this mechanism was being used more broadly when a cell synthesizes a large amount of a gene product. I had read about the unusual amino acid sequence and the huge size of the protein silk fibroin from the silkworm Bombyx mori. Most of the protein consists of repeats of the peptide glycine-alanine-glycine-alanine-glycine-serine, which predicts an mRNA and gene with a very high guanine and cytosine content. At the end of larval development, most of the protein synthesized by the posterior end of the silkworm is this single protein. At that time, only hemoglobin mRNA in reticulocytes had been identified on a sucrose gradient as a discrete mRNA. Yoshiaki Suzuki came to my lab as a postdoctoral fellow, and we began to raise silkworms. He isolated a large 32P-labeled mRNA from the posterior silkworm gland and from control silkworm tissues to show that there is a single fibroin gene in the DNA of all cell types. This ruled out DNA amplification as an explanation for the large and specific synthesis of silk fibroin mRNA by the posterior gland. Silk protein synthesis is an example of extreme translational amplification. In a period of only two to three days, each fibroin gene guides the synthesis of 10^6 copies of mRNA, each of which, in turn, synthesizes about 10^9 copies of the protein. One gene makes 10^9 protein molecules.

Amphibian metamorphosis

With the discovery of recombinant DNA technology in the late 1970s, the study of any gene became possible. In 1990, after a decade of ana-
I chose amniotic metamorphosis—a wonderful biological problem that had fascinated every succeeding biological discipline but had been ignored by molecular biologists.

The question was simple. How can one molecule—thyroid hormone—change the fate of so many different cell types? The discovery of the thyroid receptors as transcription factors by Ron Evans and Björn Vennström in 1986 convinced me that the control of metamorphosis by thyroid hormone must occur by changes in the expression of many genes.

The technical advantages of the system had been well explored. Tailpodes are exquisitely sensitive to exogenous thyroid hormone. The chemicals that inhibit synthesis of the hormone by the thyroid gland in mammals work well in amphibians. The pituitary-thyroid axis is similar in all vertebrates. The disadvantages of the system were the absence of traditional genetics and the long life cycle of frogs. When transgenesis was discovered in X. laevis in 1996, we could overexpress transgenes driven by cell-specific promoters. Among other discoveries, this enabled us to prove that the thyroid hormone receptors are responsible for all of the many changes induced by the hormone in metamorphosis. In a recent review I summarized the current state of the field.

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COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.