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In memoriam Peter H. Seeburg

John P. Adelman[1]

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During the first half of the 20th century it was shown that the brain exerts influences over endocrine glands. Research from many laboratories established that a network of small blood vessels connects the brain stem with the pituitary gland, bathing it in substances released into portal blood by the hypothalamus. Roger Guillemin and Andrew Schally, working independently, isolated hypothalamic extracts from 5 million sheep and pigs, respectively, that, when applied to pituitary tissue elicited the release of specific hormones. For example, one preparation released Thyroid Stimulating Hormone (TSH), another released Adrenocorticotropic Hormone (ACTH) and a third released the gonadotropins, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). In 1969, after an intense competition, the two groups succeeded in purifying Thyroid Releasing Hormone (TRH), a tripeptide that had a singular effect - the release of pituitary TSH. By 1971, gonadotropin releasing hormone (GnRH), a decapeptide, had been purified and in 1977 Guillemin and Schally shared the Nobel Prize in Physiology or Medicine for their work (Roger Guillemin – Biographical https://www.nobelprize.org/prizes/medicine/1977/guillemin/auto-biography/; Andrew V. Schally – Biographical https://www.nobelprize.org/prizes/medicine/1977/schally/auto-biography/), along with Rosalyn Yalow who developed radio-immuno assays of peptide hormones (Rosalyn Yalow – Facts 2018).

The work of Guillemin and Schally coincided with the birth of recombinant DNA technology and by 1979 the nascent field of molecular biology was underway. It is important to understand the state of the art at this time. There were only a few biotechnology supply companies and restriction enzymes, T4 DNA ligase, polymerases etc. were still mostly produced in-house. There were few commercially available cloning vectors, only several related plasmids that had become disseminated from the laboratories that produced them. DNA sequencing was performed using the chemical method of Maxam and Gilbert, or in a handful of laboratories, the dideoxy chain termination method of Sanger using DNA fragments cloned into the single stranded M13 phage. Oligonucleotide synthesis was performed by hand and it took a week to prepare a 15mer. This landscape would undergo a historic revolution in the early 1980s.

So it was when I met Peter Seeburg after I joined Genentech in 1980. Almost immediately we became close friends who shared an unquenchable appetite for science that made partners of a young inexperienced technician and a brilliant scientist. Our laboratory was tasked with providing clones encoding growth hormones from a variety of species in a protein expression vector to fulfill a contract with Monsanto. Despite the inefficiency of cDNA cloning at the time, growth hormone mRNA is present in very high abundance in pituitary and this task became routine. That left time to explore the frontier.

Peter introduced me to the releasing factors, masters of the endocrine system and we quickly focused on GnRH, the conductor of the reproductive orchestra. We set out to clone GnRH. What did we know? We knew for certain only the decapeptide sequence: pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. But we could make several educated guesses. The pyroGlu at the N-terminus likely results from the cyclization of the free amino group of Gln to form the lactam. Also, it was suspected that the decapeptide was embedded in a larger precursor protein that is proteolytically processed at a preceding dibasic Lys-Arg motif and that a Gly residue donates its amino group during C-terminal amidation. Still, no one had succeeded in cloning a target sequence based upon such limited information. Peter's ingenious and innovative strategy was to use degenerate pools of synthetic oligonucleotides that represented the possible coding sequence of the GnRH peptide as ^{32}P-labeled probes and identify the GnRH clone by DNA sequence analysis. As Peter's PhD thesis had been to map and define the region of M13 that regulated gene expression, one of the first identifications of a promoter, we used dideoxy Sanger sequencing.

✉ John P. Adelman
adelman@ohsu.edu

[1] Vollum Institute, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, Oregon 97239, USA

Thus, two probes were synthesized, a long probe consisting of a pool of eight oligonucleotides that spanned the decapeptide coding sequence and included three additional codons, for N-terminal Lys-Arg and C-terminal Gly and a pool of sixteen heptadecamer short probes directed to the coding sequence for the first six amino acids of the GnRH decapeptide. To validate the composition of the long probes, a short oligonucleotide directed to the 3' region primed enzymatic synthesis of double stranded products that were cloned into M13. DNA sequencing of many clones verified the relative proportions of the probe pool. Because we anticipated that the GnRH mRNA would be expressed at relatively low levels in the hypothalamus and might contain a long 3' untranslated domain, compromising representation of the decapeptide coding sequence in cDNA, we decided to screen a human genomic library, aware of the caveat that an intron might disrupt the GnRH coding sequence. To engender confidence that our probes would work as expected, we cloned members of the probe pool into lambda phage and mixed this control sequence phage into the library at several dilutions. Centrally important, this also allowed us to determine and optimize hybridization and wash stringencies. For the large-scale screen of 10^6 phage clones that should represent all genomic sequences, the long probes detected ~300 clones with a wide range of signal intensities. When these long probe-positive clones were rescreened with the long and the short probes, a single clone hybridized with both probes. Restriction digest and Southern blot analysis of the clone showed that the same fragments hybridized to the two sets of probes, and DNA sequence analysis determined that the clone did indeed encode GnRH. While there were several mismatches with our probes, we had determined effective screening parameters. The coding sequence showed a C-terminal Gly, as we had anticipated, followed by a Lys-Arg processing site, however there was no such processing site at the N-terminal coding sequence.

During the ~2 years that the work described above was performed, Peter and I had been working to construct high complexity cDNA libraries. Again, Peter's genius conceived the methodology to synthesize complementary strands, size select the cDNA, ligate synthetic adaptors with built in restriction sites and use lambda phage vectors. Thus, by the time we had isolated the genomic clone we were poised to screen for the corresponding cDNA. For this purpose, we chose to construct a placental cDNA library as there were reports of GnRH (and almost every other peptide) expression in placenta and human hypothalamus was not available to us. Using a long fragment of the genomic clone as a probe, we isolated the corresponding GnRH cDNA, revealing for the first time, the structure of pre-proGnRH. There is a 23 amino acid signal peptide followed by Ser-Ser and then the GnRH coding sequence and the expected C-terminal Gly-Lys-Arg. The remainder of the prohormone consists of a novel 56 amino acid protein that was subsequently shown to be involved in regulating prolactin secretion (Seeburg and Adelman 1984).

In addition, we synthesized cDNA libraries from human and rat hypothalamus and isolated cDNA clones encoding pre-proGnRH. This showed that the same precursor is expressed in hypothalamus and placenta (Adelman et al. 1986). We also determined the sequence and structure for the entire genomic locus of the single GnRH gene in mammals (Hayflick et al. 1989) and we described the first long non-coding RNAs derived from the opposite DNA strand of the GnRH locus (Adelman et al. 1987).

There are several other people who contributed to our success in this project. Kim Dinkelspiel had magic hands for synthetic DNA chemistry and provided us with the probes that were instrumental to our success. Dave Goeddel, the Chair of our department, appreciated that Peter and I could provide technical advances that would benefit the rest of Genentech and therefore, allowed us to spend a great deal of time working on GnRH that had no pharmaceutical value. Karoly Nikolics who came as a postdoctoral fellow inherited the clones and discovered the function of Prolactin Release-inhibiting Factor that cohabits pre-proGnRH (Nikolics et al. 1985). Karoly remained Peter's lifelong friend. Joel Hayflick and Ellson Chen advanced DNA sequencing technology.

When I reflect on this formative time, the first thing I appreciate is Peter's intellect. His insights and strategies were nothing short of genius. I am also fortunate to have worked at the bench with Peter because his intellect was paralleled by an astonishing technical ability. At a time when I knew I could do anything at the bench, watching Peter work was humbling. I was also privileged to have been so close to Peter for so many years. He was a masterful mentor, teaching me how to think science, how to write and importantly to be absolutely driven yet humble. He was a beautiful colleague and always my friend.

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