3. *IN VIVO* ELECTROPORATION METHODOLOGIES TO STUDY MALE REPRODUCTIVE GENE EXPRESSION

J.L. Kirby, R.J. Lye, J.C. Labus and B.T. Hinton
Department of Cell Biology, University of Virginia Health System, School of Medicine, Charlottesville, VA 22911, USA

For the past several years our laboratory has been interested in the "lumicrine" regulation of epididymal gene expression by testicular luminal growth factors. To understand the *in vivo* regulation of epididymal genes via this mode of regulation we have used a modified *in vivo* electroporation technique (1). Initial studies were performed with a construct containing EGFP under the control of the CMV promoter to determine the optimal conditions for experiments in the rat epididymis. Approximately 20 μL containing 1–4 μg of construct DNA was microinjected into the lumen and/or interstitium of different epididymal regions using micropuncture techniques. Following injection, the DNA was electroporated into the cells of the epididymis using a pair of tweezer electrodes in conjunction with a BTX 830 electroporator. The electrodes were kept at a constant distance of 0.2 cm to deliver 8 pulses of 21–24 V/50 msec pulse. Following an appropriate time interval, the tissue was removed and analyzed for EGFP fluorescence. The initial segment clearly showed expression of EGFP in every epithelial cell in the region of the electrodes. The electroporation technique was used to perform an *in vivo* promoter analysis of gamma-glutamyl transpeptidase mRNA IV promoter, a gene specifically expressed in the rat initial segment, and electroporated various dominant negative and activating mutant constructs to dissect the FGF/FGFR-MAPK signal transduction pathway within the initial segment of the rat epididymis. This technique has also proved useful for testing "knockout" constructs for biological activity and gene silencing using RNAi and antisense morpholinos.

Supported by NIH-NICHD HD 32979, Ernst Schering Research Foundation and The Rockefeller Foundation.

(1) Muramatsu, T., Shibata, O., Ryoki, S., Ohmori, Y. and Okumura, J-I. (1997). *Biochem. Biophys. Res. Commun*., **233**, 45–49.