DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Alcohol, Drug Abuse, and
Mental Health Administration
National Institute on Drug Abuse
Addiction Research Center
P.O. Box 5180
Baltimore MD 21224

January 10, 1992

Dr. Daniel Nathans
Department of Molecular Biology & Genetics
Johns Hopkins University Sch. of Medicine
725 N. Wolfe Street
Baltimore, Maryland 21205-2185

Dear Dr. Nathans:

Three months ago I received from your laboratory cDNA probes for Zif 268 and FOS B. Since then I have performed several experiments on Northern blots with total or poly(A)$^+$ mRNA extracted from amphetamine- and saline-treated 7-8 weeks old Sprague Dawley rats. The results I observed using the FOS B probe indicate it highlights a message that responds like cFOS to drug treatment, but has a different size (approximately 4.0 kb) than the 5.1 kb size reported by Zerial et al. and confirmed by Nakabeppu, Y. and Nathans, D. I would thus like to briefly describe to you the reasons for my perplexity regarding FOS B message size.

Zerial et al. (EMBO Journal-1989) describe a FOS B 5.1 kb mRNA message observed in stimulated NIH 3D3 cells. Yet the Northern blots presented in Figure 4 (which I have copied for your convenience) display a FOS B message that runs faster than the 28S rRNA. I have so far hybridized 5 blots with your probe, which hybridizes each time to a fragment located between 28S and 18S, in a position practically identical to that displayed by FOS B message in Figure 4 of Zerial et al. If we are to believe that 28S runs at 4.8 kb and 18S at 1.7 kb, it is difficult for me to see how this message could be 5.1 kb. Furthermore, a 3.9-4.0 kb estimate is confirmed also on 2 blots where I ran RNA ladders.

My first worry at this point was to insure that the probe that I was using was indeed the FOS B insert you had sent me, cloned in pBluescript KS$^-$. As you can see on the photo I am including, a digest with Eco, Pst and Bam gave fragments of the expected size (bright bands),though suggesting the presence of a very minor contamination (faint bands). Furthermore, none of the probes I had previously used from those same blots, namely, cFOS and beta-actin, gives bands overlapping with my "putative" FOS B (see last figure). Finally, as you can barely see on this figure (I apologize for the quality of the copy), FOS B expression is significantly activated by acute amphetamine administration, with a time course which is identical to that of cFOS.

In conclusion, I think I am using the right insert, what I detect behaves like cFOS and these FOS B bands located between 28S and 18S like in figure 4 of Zerial et al. are not "left-overs" from previous hybridization. In your paper "A Naturally Occurring Truncated Form of FosB That Inhibits Fos/Jun Transcription Activity," you report Northern blots data from several tissues confirming a 5 kb

message size. About one month ago I have spoken with Laura Sanders, who told me she had done a very small amount of work on the FOS B project and suggested I should contact you to ask your opinion on the matter.

I hope you will forgive me for stealing a bit of your time for an apparently minor issue, but I cannot come up with a valid explanation. Yet, an answer to this question is necessary, before I proceed with my work. I would thus very much appreciate your suggestions. If you prefer to contact me by phone, rather than by mail, I can be reached in Baltimore at 550-1464 (fax: 550-1645).

Thank you very much for your help.

Sincerely,

Antonio M. Persico, M.D.
Lab. of Mol. Neurobiology
NIDA/ARC