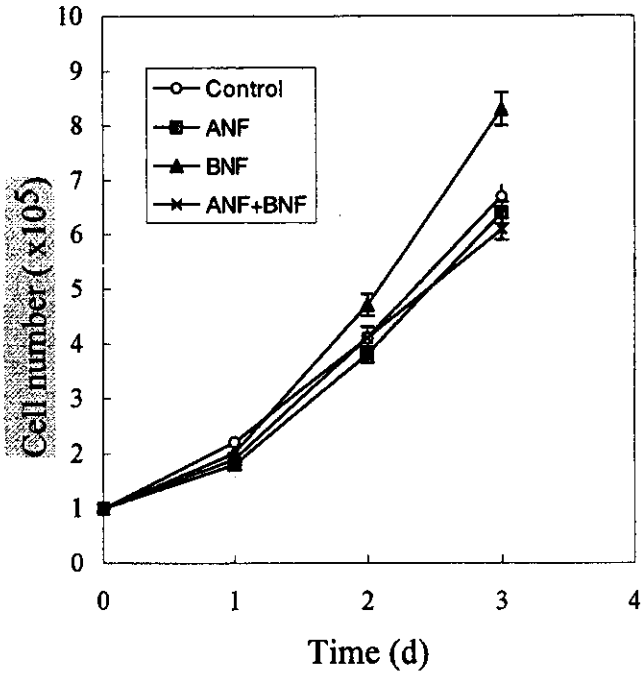

図1 Effect of AhR ligands on cell proliferation in A549 cells.

A549 cells were treated with 10 μM β-naphthoflavone (BNF) or 10 μM α-naphthoflavone (ANF). The AhR ligands were dissolved in dimethylsulfoxide (DMSO), and control cells were given the same amounts (2 μl) of DMSO. Cell numbers were determined by hemocytometer cell counts. Each time point represents a triplicate determination, and each experiment was performed twice with similar results. Symbols are defined in the figure.

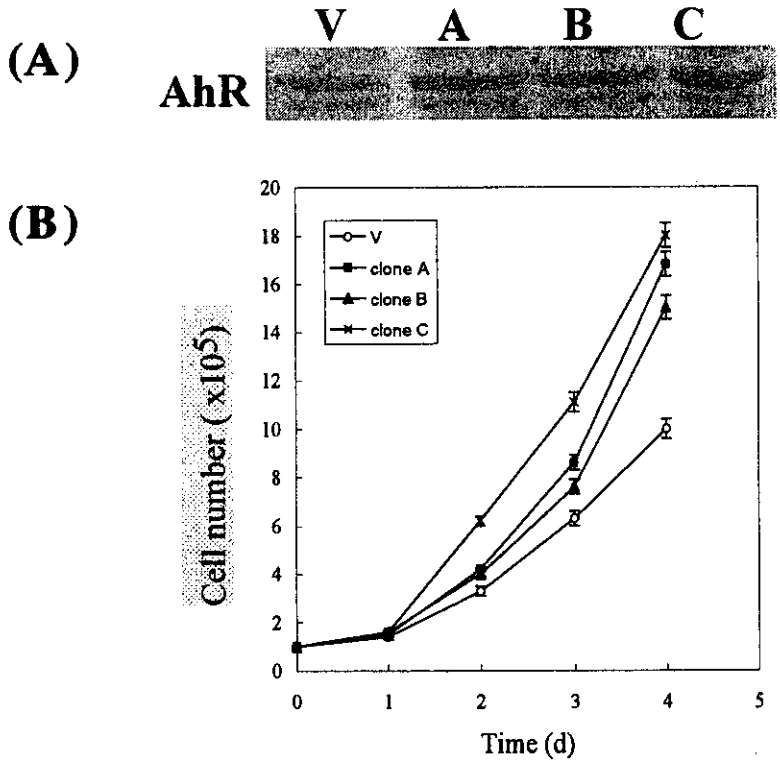

図2 . Overexpression of the AhR accelerates cell proliferation in A549 cells.

(A) Western blot analysis of the AhR and the actin was performed on whole cell extracts (5μg of protein) resolved by electrophoresis on 10% SDS/polyacrylamide gel.
V: cells expressing the vector mRNA.
(B) Cell growth of the clones was measured as described in the legend for Fig. 1. Data represent means of determinations made on three independent dishes. The analysis was reproduced twice with similar results. Symbols are defined in the figure.

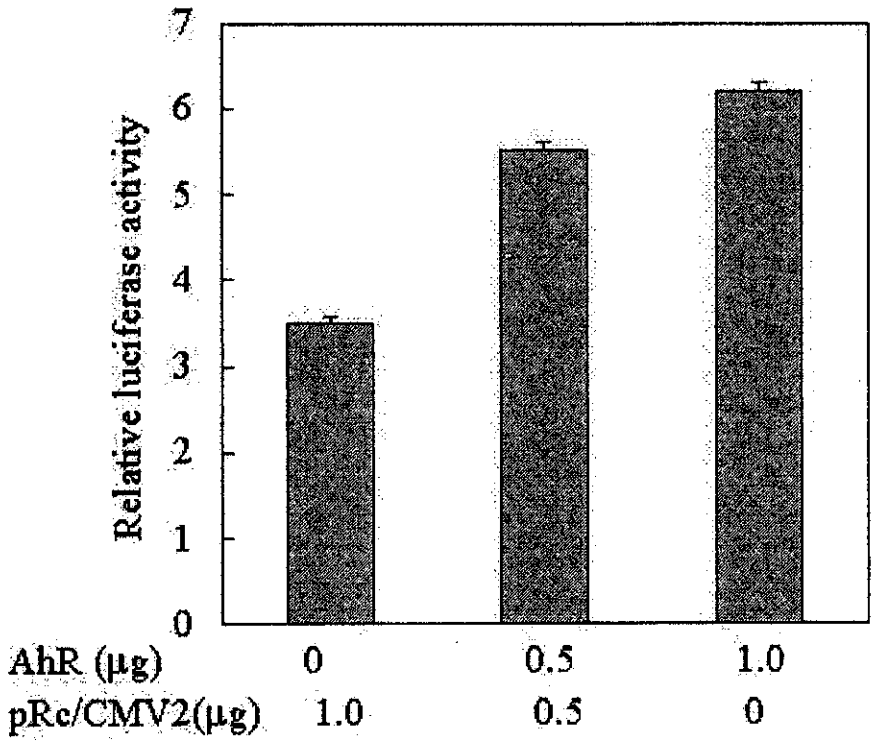

図3　**The AhR enhances E2F binding activity in A549 cells.**
Increasing amounts of the AhR expression vector were transfected into A549 cells with the reporter plasmid E2F/luciferase. For all transfection, pRL-SV40 control vector was cotransfected to correct for differences in transfection efficiency. Data represent means of determinations made on three independent dishes.

Dual-Luciferase Reporter Assay Kit (Promega) を用いて測定した。

C. 結果・考察

AhRのリガンドのひとつであるbNFはA549細胞の増殖を促進した（図1）。AhR量が異なるいくつかの細胞クローンの増殖速度を検討したところ、AhR量に比例して細胞増殖速度は増加した（図2）。細胞をM期同調し、その後のS期への以降を検討したところAhR過剰発現株はコントロール細胞に先立ってS期へと移行した。DNAアレイを用いた検討によりAhRの活性化がE2Fの標的遺伝子群の発現を増加させることが示された。また、リポーター遺伝子を用いた検討よりAhRの活性化はE2F依存的転写活性を増加させることが示された（図3）。以上の結果よりAhRがE2Fとのクロストークを介してA549細胞における細胞増殖に関与することが示唆された。