THE CHARACTERIZATION OF FIBROBLAST GROWTH FACTOR
AND ITS BIOLOGICAL EFFECT IN VITRO AND IN VIVO

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1. INTRODUCTION

Over the last 4 years, both basic and acidic Fibroblast growth factors (bFGF and aFGF) have been purified to homogeneity, their primary structures determined, and their cDNA cloned and sequenced (reviewed in ref.1-3). This information has had significant impact on our understanding of a variety of mitogenic activities isolated from diverse origin. It has become clear that growth factors isolated from ovary, adrenal, kidney, eye, brain, placenta, macrophages, prostate, cartilage, and various tumors, are structurally and biologically identical, or at least, very similar to bFGF or aFGF (reviewed in ref.1). Availability of the pure mitogens has led to the recognition of a wide spectrum of activities for these two factors (4), most notably, their ability to mimic the biological effect of the vegetalizing factor in early embryos (5) and to act as angiogenic factors. Basic FGF or aFGF are multifunctional, since they can both stimulate proliferation and induce or delay differentiation. They stimulate other critical processes in cell function as well. Thus far, so many new and varied functions have now been described for FGF's that at present, one must consider these peptides to be of special importance for the control of cell growth and differentiation.

2. PRIMARY STRUCTURE, GENOMIC ORGANIZATION, AND mRNA EXPRESSION
OF ACIDIC AND BASIC FGF

Basic FGF has been purified from most mesoderm- or neuroectoderm-derived tissues or cells which have in common a strong angiogenic potential (1,2). Structural studies have shown that bFGF is a single chain peptide composed of 146 amino acids (6) which can also exist in an NH2-terminally truncated form missing the first 15 amino acids (7). The truncated form of bFGF is as potent as native bFGF, as demonstrated by radioreceptor binding and biological assays, indicating that the NH2-terminal region of bFGF is neither involved in its binding to FGF cell surface receptors nor in its biological activity (8). Related to bFGF is aFGF, which shares a 55% total sequence homology with bFGF (9). Acidic FGF is a 140-amino acid peptide which can also exist as an NH2-terminally truncated form missing the first 6 amino acids (10). The high degree of homology between aFGF and bFGF suggests that they are derived from a common ancestral gene.

Evidence that a viral oncogene may code for a growth factor or part of a growth factor receptor has recently emerged from studies on the PDGF structure and that of the EGF receptor structure (reviewed in ref.11). In the case of basic FGF, a 46% and 42.3% homology, respectively, has been shown to exist with the predicted product of int-2 and the hst gene product (12,13). A lesser degree of structural

homology exists between those two gene products and aFGF. While Int-2 has been implicated in the induction of virally induced mammary cancer (14), the hst gene was originally identified as a transforming gene in DNA's from human stomach cancer (15).

The FGF genes have been cloned and complementary DNA sequences of both bFGF and aFGF have been synthesized. The genomic organization of the genes encoding bFGF and aFGF has been described (16,17,18). The bFGF gene is localized on human chromosome 4, while that of aFGF is on chromosome 5 (3). This suggests that through a process of gene duplication and evolutionary divergence, bFGF and aFGF have become separate gene products. The basic FGF gene, with its size greater than 38 kbp, encodes two exons widely separated by two introns: the first one separates codons 60 and 61, and the second separates codons 94 and 95. The aFGF gene has a similar organization, with 2 large introns located in identical positions in the coding sequence once basic and aFGF are properly aligned. Southern blot analysis of human genomic DNA has shown that there is only one bFGF and one aFGF gene. Therefore, all of the characterized or uncharacterized heparin-binding endothelial cell mitogens related to bFGF or aFGF are the products of a single bFGF or aFGF gene (3,16). In various cultured cells and tissues, the bFGF gene gives rise to two polyadenylated mRNA's of approximately 3.7 and 7.0 kb (reviewed in ref.19). The aFGF gene appears to encode a single mRNA species of approximately 4.1 kb (20). The primary translation product for either bFGF or aFGF is composed of 155 amino acids. Proteolytic cleavage from the precursor molecule of the first 9 (bFGF) or 15 residues (aFGF) would result in the generation of the mature proteins which can then be cleaved further in homologous positions to give the NH₂-truncated form of bFGF (des.1-15) or aFGF (des.1-6) (reviewed in ref.3,19).

3. bFGF GENE EXPRESSION IN CELL TYPES WHICH DO NOT EXPRESS THE bFGF GENE, BUT RESPOND TO bFGF, RESULTS IN AUTONOMOUS CELL PROLIFERATION

The concept of autocrine stimulation of cell proliferation postulates that normal diploid cells can gain growth autonomy by acquiring the ability to produce, secrete, and respond to a given growth factor (21,22). Verification of the autocrine hypothesis, in the case of bFGF, requires demonstration that expression of an introduced FGF gene in non-tumorigenic cells results in or contributes to the malignant transformation of those cells.

The hypothesis that inappropriate expression of bFGF could lead to cell transformation, has been tested by introducing into BHK-21 cells a plasmid that directs the high-level expression of human bFGF. BHK-21 cells were chosen because they do not express the bFGF gene, and in previous studies, they have been shown to be totally dependent on exogenous bFGF in order to proliferate when maintained under serum-free conditions (23). Finally, exogenous bFGF induces anchorage-independent soft agar growth of BHK-21 cells. This effect, however, is transient, and cells revert to their normal phenotype once the mitogen is removed. High-level bFGF expression in BHK-21 cells might therefore be expected to lead to permanent anchorage-independent soft agar growth of BHK-21 cells.

Southern blot analysis demonstrates that in BHK-21 cells, following transfection, at least one complete bFGF gene copy per cell has been stably incorporated; a second, partial copy also appears to be present. That the integrated foreign bFGF genes are actively expressed