Basic Fibroblast Growth Factor-Like Substance in Nuclei of Male Germ Cells Undergoing Meiosis (43310)

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Abstract. **The presence of a basic fibroblast growth factor-like immunoreactive substance was demonstrated in the nuclei of germ cells at stages from spermatocyte to spermatid in adult rat testis by using immunohistochemistry with an antibody raised against a synthetic peptide corresponding to residues 1–10 of bovine basic fibroblast growth factor [1-146]. The fluorescence was very weak in the nuclei and cytoplasm of spermatogonia, Sertoli cells, and most of the interstitial compartments, except for capillary endothelial cells. This is the first study to demonstrate the presence of basic fibroblast growth factor-like immunoreactive material in the nuclei of haploid cells *in vivo*.** [P.S.E.B.M. 1991, Vol 198]

Basic fibroblast growth factor (FGF) has been assumed to be identical to basic heparin-binding growth factor (1, 2), and other basic growth factors derived from various organs (3–10). It has been extracted from a number of tissues, including testis tissue (10). The major physiological role of basic FGF is thought to be angiogenesis, in view of its stimulatory activity on endothelial cell proliferation and mobility (11). Basic FGF has also been found to be mitogenic for a variety of normal diploid cell types of mesodermal and neural crest cell origin (12). Ueno *et al.* (10) suggested that basic FGF might regulate the proliferation of germ cells and the differentiation of the gonad. It is known that cultured Sertoli cells contain a FGF-like factor (13). More recently, basic FGF was detected in both the cytoplasm and the nuclei of various types of cultured somatic cells by immunohistochemistry (14, 15). Basic FGF was originally described as a polypeptide with an apparent molecular mass of 16.5 kDa (146 amino acids) which was isolated from bovine pituitary and brain (7, 8). Analysis of the cDNA sequence of basic FGF (1) suggested that the translation product was, in fact, 154 amino acids long and, thus, at least 18 kDa in size. The 154-amino acid protein was subsequently purified in the presence of protease inhibitors (16, 17).

We have been interested in the role of basic FGF in a mutant strain of rat which may lack an unknown factor necessary for testicular development (18–20). The present study demonstrated the presence of basic FGF-like immunoreactive material in the nuclei of germ cells in adult rat testis.

Materials and Methods

Antibodies. The polyclonal antibody used in this study was raised in a rabbit against a 1–10 (PALPEDGGSG) synthetic fragment of bovine basic FGF [1-146] conjugated to bovine thyroglobulin. This polyclonal antibody showed no cross-reactivity with acidic FGF. In Western blots of heparin affinity-purified extracts of rat testis, the antibody yielded a single band at 16.5 kDa, corresponding to the molecular mass of basic FGF (which will appear elsewhere). Although its cross-reactivity with other members of the FGF family (21), int-2, hst, and FGF-5, was not tested, all have low

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sequence homology in the areas corresponding to the sequence of the peptide antigen used. Fluorescein-5-isothiocyanate-labeled goat anti-rabbit IgG was purchased from Cappel, Organon Teknica Corp. (West Chester, PA) and used as the second antibody.

Tissue Preparation and Immunohistochemistry. Normal adult male rats (aged 70 days) of the Wistar-Imamichi strain, maintained as a closed colony at the Department of Veterinary Physiology, Nippon Veterinary and Animal Science University (22), were used. The testis, removed under light ether anesthesia, was embedded in Tissue-Tek O.C.T. compound (Miles Scientific, Naperville, IL) and frozen in dry ice and acetone. Frozen sections 5-μm thick were incubated with antibody raised against a 1–10 synthetic fragment of bovine basic FGF [1-146], and then incubated with the secondary fluorescein-5-isothiocyanate-labeled antirabbit IgG antibody and photographed using a fluorescence and phase-contrast microscope. The stages of the cycle of the seminiferous epithelium were identified according to the well-established morphologic criteria (23). The first antibody absorbed by the whole molecule or the 1–10 synthetic fragment was used as the control. The intact basic FGF was of bovine brain origin and was purchased from Funakoshi Chemical Co. Ltd., Tokyo, Japan. Photographs were taken using a Nikon BVD fluorescence microscope, and phase-contrast figures were also obtained.

Results

The localization of fluorescence in the testis is shown in the micrographs in Figure 1. In adult testis, the nuclei of the primary and secondary spermatocytes and spermatids showed strong fluorescence (Fig. 1, A and B), but the Step 19 spermatids of the seminiferous epithelium at Stage VIII of the cycle did not (Fig. 1A). Sertoli and myoid cells showed implausible fluorescence. The spermatogonia showed positive reaction (Fig. 1C). In the interstitial compartments, vascular endothelial cells showed marked fluorescence, and some oval-shaped cells had weakly fluorescent cytoplasm.

Neutralization of the antibody by the synthetic fragment or whole molecule of FGF completely abolished the fluorescence in the germ and endothelial cells (Fig. 1, E and F). Therefore, it is considered that a basic FGF-like immunoreactive substance is located where the fluorescence was detected when anti-basic FGF antibody alone was used. When the whole basic FGF molecule was used for neutralization, a marked reaction was detected in the basement membranes of the seminiferous tubules and tunica intima of the arteries (Fig. 1E). This would be due to the binding of antigen-antibody complex to these elements, and indicates that the heparin-binding domains of basic FGF remain intact after binding of the N-terminal to the antibody. These findings are in accord with evidence that basic FGF has two sequences (residues 18–22 and 107–110 of bovine pituitary basic FGF) capable of binding to heparin or heparan sulfate proteoglycans (7, 24, 25). Furthermore, negativity was also confirmed without primary antibody and positivity was confirmed in the pituitary gland, which was one of the known sources of basic FGF (data not shown).

Discussion

The present study is the first to demonstrate the histological localization of basic FGF-like immunoreactivity in the nuclei of haploid cells. Since basic FGF does not have classical signal sequences in its molecule (1), it is difficult to determine whether its mode of secretion is paracrine or autocrine. It has been reported recently that cell destruction might release basic FGF from the cytoplasm into the extracellular matrix, where basic FGF would bind to heparin and then pass through the membrane of target cells (3, 24, 26, 27).

In the reproductive system, basic FGF has been purified from bovine corpus luteum (9), granulosa cells (28), and bovine and human testes (10, 29). Immunohistochemical localization of basic FGF has also been revealed in 18-day rat fetal testis and ovary (30), and it has been shown that cultured Sertoli cells contain an FGF-like factor (13). A regulatory effect of basic FGF on both testosterone secretion (31) and testicular aromatase activity has been found in cultured immature porcine Leydig cells (32). These findings suggest that basic FGF may play a role in the local control of testicular steroidogenesis, and that it is important for Sertoli or Leydig cell function.

Bouche *et al.* (33) have shown that basic FGF enters the nucleolus and stimulates the transcription of ribosomal genes in adult bovine aortic endothelial cells undergoing G_0 to G_1 transition. They also obtained immunocytochemical evidence for the accumulation of basic FGF in the nucleolus of these cells. More recently, basic FGF was detected immunocytochemically in both the cytoplasm and nuclei of endothelial cells known to produce basic FGF and BHK-21. SK-Hep-1, or NIH 3T3 cells transfected with basic FGF cDNA (14, 15). It has been reported (11, 34, 35) that protamine, showing strong affinity for heparin, inhibits mast cell locomotion, whereas heparin stimulates the locomotion of endothelial cells (34) and also inhibits angiogenesis associated with embryogenesis, inflammation, and certain immune reactions (35). Nuclear histone (somatic and testis-specific types) in spermatocytes has been reported to be transformed to protamine during the final steps of spermiogenesis (36). Histones are present in Steps 1–8 spermatids, but are absent after Step 12 (37) and are replaced by nuclear transition proteins designated TP1 and TP2, characteristic to Steps 13–15 (37). These are replaced in turn during Steps 16–19 by

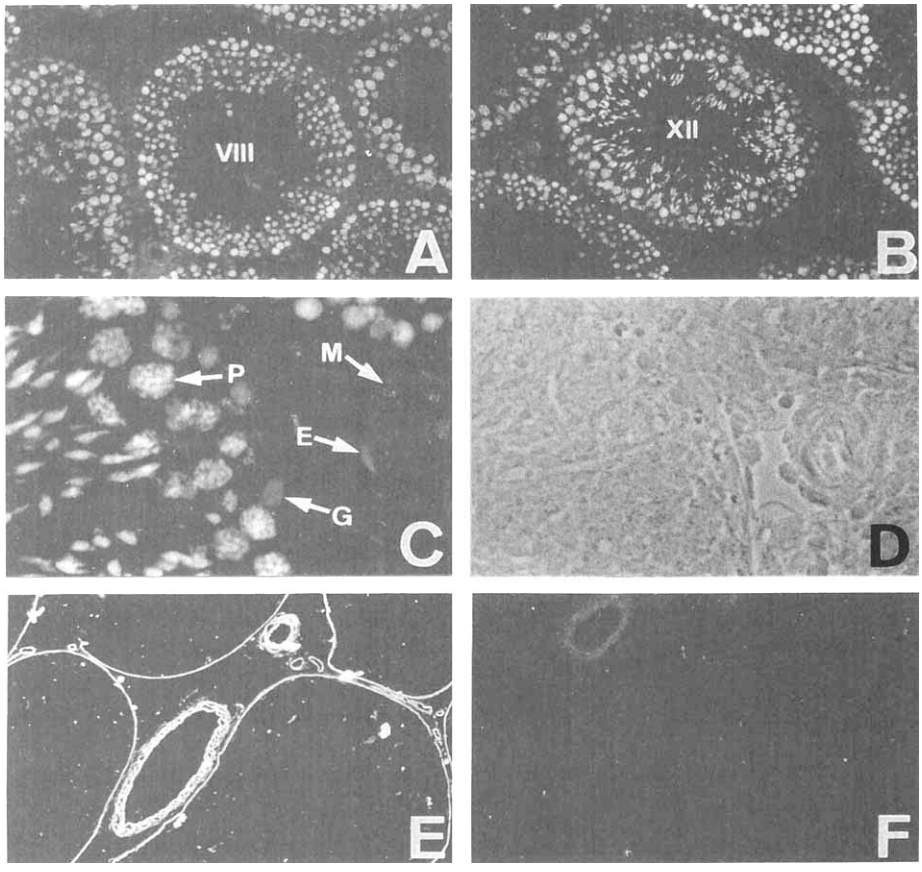

Figure 1. Immunofluorescence localization of basic FGF-like immunoreactive material in adult rat testis. (A) Seminiferous epithelium at Stage VIII of the cycle; indirect immunofluorescence using anti-bovine basic FGF (1–10) antibody (original magnification ×100). (B) Seminiferous epithelium at Stage XII of the cycle; indirect immunofluorescence using anti-bovine basic FGF (1–10) antibody (original magnification ×100). (C) Higher magnification of B (original magnification ×400). Abbreviations used in C: G, spermatogonia; E, endothelial cell; P, pachytene primary spermatocyte; M, myoid cell. The endothelial cells show very weak immunoreactivity in this field. (D) Phase-contrast microscopy of C (original magnification ×400). (E) Indirect immunofluorescence using anti-bovine basic FGF (1–10) antibody preabsorbed with the whole molecule of basic FGF (original magnification ×100). (F) Indirect immunofluorescence using anti-bovine basic FGF (1–10) antibody preabsorbed with a 1–10 synthetic fragment of bovine basic FGF (1–146) (original magnification ×100).

basic nuclear protein TP3 and by sperm basic nuclear protein S1 (37). These changes in protein composition seem to be correlated with the presence of basic FGF-like immunoreactivity in germ cell nuclei containing histones. Therefore, protamine might act on the basic FGF-DNA-heparin complex to delete basic FGF. This process would be especially important in the later period of spermatid development, and such cells did not show fluorescence in the present study.

The fluorescence observed in our study seemed to correspond to the arrangement of chromatin or chromosomes. In contrast, only minor basic FGF-like immunoreactivity was detected in the cytoplasm of the interstitial compartment and in some cells in the seminiferous tubules. The predominance of basic FGF-like immunoreactivity in the nuclei of germ cells undergoing meiosis appears to suggest a novel biological role of basic FGF in gametogenesis. Our findings in haploid cells during meiosis seem completely different from those in diploid cells, and this may also suggest direct specific or nonspecific binding between basic FGF-like immunoreactive material and DNA. Further research will be needed to clarify how, where, and when the basic FGF or basic FGF-like immunoreactive material is synthesized and carried into the nucleus.

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