Development 111, 1057–1060 (1991)
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# IGF-2 receptors are first expressed at the 2-cell stage of mouse development

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## Summary

**A specific IGF-2 receptor antiserum was used to reveal the presence of IGF-2 receptors during preimplantation development of mice. Receptors were present on 2-, 4- and 8-cell embryos, morulae, blastocysts, and on ICMs isolated prior to staining. There was no evidence for receptors on fertilized eggs. These observations confirm reports of the expression of IGF-2 receptor mRNA as early as the 2-cell stage and refine similar observations in blastocysts to confirm expression in both the TE and ICM. A potential auto/paracrine loop is thus one of the first products of activation of the embryonic genome and is expressed constitutively through preimplantation development.**

Key words: IGF-2, receptor, preimplantation embryo, immunohistochemistry.

## Introduction

Insulin and insulin-like growth factors -1 and -2 (IGF-1, -2) promote metabolism and growth in many cell types (Froesch and Zapf, 1985). In the preimplantation mouse embryo, both insulin (Gardner and Kaye, 1991; Harvey and Kaye, 1990*a*) and IGF-1 (Harvey and Kaye, 1989) stimulate cell division and blastocyst formation *in vitro via* their own receptors.

However, IGF-2 has been suggested as the specific embryonic/fetal member of this growth factor family (Moses *et al.* 1980). Although displacement of insulin from receptors by a mixture of IGFs-1 and -2 (Mattson *et al.* 1988) and demonstration of IGF-2 receptor mRNA in compacted embryos have been reported, the actual time of first expression of this mRNA has been placed between the 2-cell (Rappolee *et al.* 1989*a*; Werb, 1990) and 8-cell stages (Heyner *et al.* 1989; Rappolee *et al.* 1989*b*). On the other hand, IGF-2 mRNA has been revealed in 2-cell embryos (Heyner *et al.* 1989).

In an attempt to define the ontogeny of the IGF-2 receptor (or type 2 mannose-6-phosphate receptor) and thus the potential for auto/paracrine regulation at this early developmental stage, a specific IGF-2 receptor antibody (Scott and Baxter, 1987) has been used to define the expression of the receptor in embryonic cell membranes immunohistochemically.

## Materials and methods

*Superovulation and Embryo Manipulation*

Randomly bred, Quackenbush mice (10 weeks) were superovulated by *i.p.* injection of 10 i.u. PMSG at 10.00 h followed 48 h later by 10 i.u. hCG (Folligon and Chorulon, Intervet, Aust.) and paired with males. Mating was determined by the presence of a vaginal plug by 9.00 h the following morning.

*Immunohistochemistry*

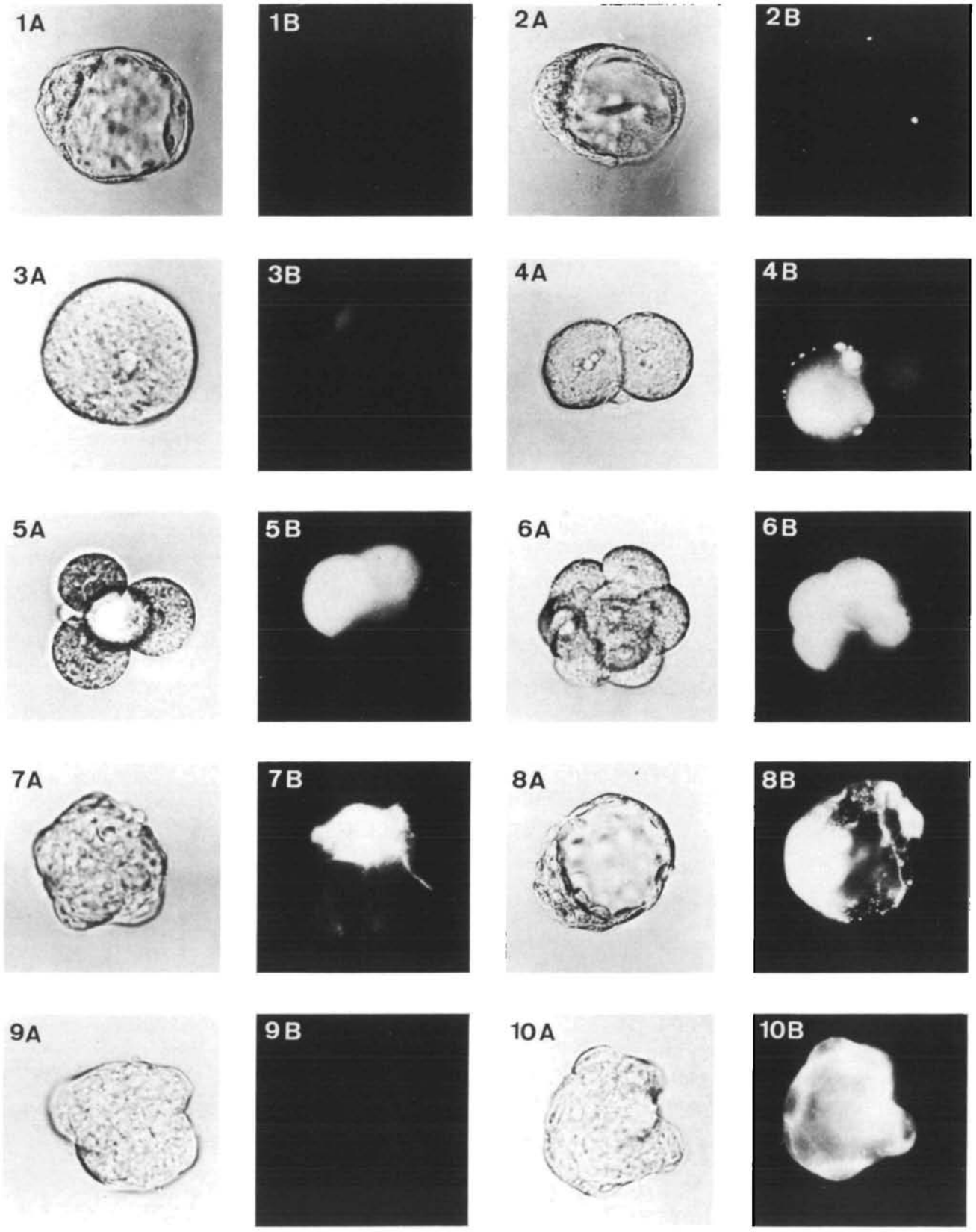
Fertilized eggs, 2-, 4- and 8-cell embryos, and blastocysts were collected 24 h, 48 h, 60 h, 72 h, and 96 h post-hCG respectively in M2 (Fulton and Whittingham, 1978; modified as previously described, Hobbs and Kaye, 1985). IGF-2 receptors were visualized by modification of the procedure used for insulin receptors (Harvey and Kaye, 1991). Briefly, the zona pellucidae were removed by brief exposure to acid Tyrode's solution (pH 2.5; Hogan *et al.* 1986), before fixation with 2% paraformaldehyde in 10 mM phosphate buffer (pH 7.2) and transferred to 1:600 dilution of rabbit anti-rat IGF-2 receptor IgG (Scott and Baxter, 1987) or control rabbit IgG (1:600 dilution). After 90 min, embryos were washed and the bound IGF-2 receptor IgG 'tagged' with a biotinylated, donkey anti-rabbit IgG (1:200 dilution; Amersham Aust. Pty Ltd, Nth Ryde, NSW, Aust.) and streptavidin-linked Texas red complex (1:100 dilution; Amersham Aust.). Embryos were then mounted in glycerol and visualized under a photomicroscope.

*ICM Isolation*

ICMs were isolated from blastocysts by immunosurgery (Solter and Knowles, 1975; Harvey and Kaye, 1991).

## Results

Blastocysts that are reported to express IGF-2 receptor mRNA (Rappolee *et al.* 1989*a*,*b*) and from which




**Figs 1–10.** Fixed and stained mouse preimplantation embryos (A) light microscopy (B) fluorescence microscopy; (Fig. 1 and 9) blastocyst (×250) and isolated ICM (×400), respectively, incubated with control IgG (Fig. 2) blastocyst incubated with 130 $\mu$M IGF-2 prior and during IGF-2 receptor IgG incubation (Fig. 3–8, 10) fertilized egg, 2-, 4- and 8-cell embryo, morulae, blastocyst (×250) and isolated ICM (×400), respectively, incubated with IGF-2 receptor IgG.

insulin can be displaced by IGF-2 (Mattson *et al.* 1988) and whose metabolism was stimulated by IGF-2 (Rappolee *et al.* 1989*a*) were used as controls to confirm the procedure.

No staining was present on blastocysts or isolated ICMs incubated with the control rabbit IgG (Figs 1, 9) or on fertilized eggs (Fig. 3). IGF-2 receptors were visible on 2-, 4- and 8-cell embryos, morulae, blastocysts and isolated ICMs (Figs 4,5,6,7,8,10). Consistent staining was seen on embryos at these stages from at least 3 experiments. In the majority of early cleavage stage embryos observed, there was a greater degree of staining on approximately 50% of the blastomeres present (Figs 4,5,6). The specificity of this staining to IGF-2 receptors was demonstrated by the absence of staining on blastocysts when the primary antibody incubation was omitted (data not shown) or a marked decrease in fluorescence on blastocysts incubated with 130 $\mu$M IGF-2 (Lilly Research Laboratories, Indianapolis, Indiana USA) prior to fixation, and before and during IGF-2 receptor IgG incubation (Fig. 2).

## Discussion

The aim of this study was to investigate if, and when, IGF-2 receptors appeared on embryonic cell membranes during preimplantation development and on which cell population of the blastocyst they were expressed. The specificity of the receptor antibody for the IGF-2 receptor used in this study has been established by the complete abolition of specific staining on hepatocytes and hepatoma cells following preincubation of the antiserum with purified rat type 2 receptor and by its reaction with a single species of relative molecular mass 210000–220000 (consistent with that of the IGF-2 receptor) when liver and hepatoma cell micosomal membranes were western blotted after electrophoresis (Hartshorn *et al.* 1989). This antibody has also been used to show specific IGF-2 receptor staining on developing mouse sceral cells (Cuthbertson *et al.* 1989).

Staining was present on embryos from the 2-cell stage through to blastocysts (Figs 4–8), correlating with the presence of IGF-2 receptor mRNA in 2-cell and later stage embryos detected using reverse transcription and polymerase chain reaction (Rappolee *et al.* 1989*a*,*b*). As there is no other evidence for the presence of insulin or IGF-1 receptors prior to the 8-cell stage (Rappolee *et al.* 1989*a*,*b*; Harvey and Kaye, 1988, 1990, 1991) and these ligands may cross react with IGF-2 receptors (Froesch and Zapf, 1985), the early appearance of IGF-2 receptors at the 2-cell stage may explain the early growth effects of insulin (Gardner and Kaye, 1991) and IGF-1 (Harvey and Kaye, 1989).

Tight junctions between cells of the TE prevent access of antibodies to the ICM (Harvey and Kaye, 1991), so the staining on compacted embryos reveals the presence of IGF-2 receptors only on the outer TE cells. Immunosurgical isolation of ICMs prior to fixation and staining (Fig. 10) confirmed the presence of IGF-2 receptors on this cell population. It is thus apparent that IGF-2 receptors are present from the later 2-cell stage until the first differentiation which occurs at compaction (at the 8-cell stage) and are then expressed in both newly differentiated cell populations.

This ontogeny of the IGF-2 receptor supports the suggestion that it may be a product of the embryonic genome (Werb, 1990) that is activated in the 2-cell stage (Flach *et al.* 1982) as receptors were not evident on fertilized eggs (Fig. 3). As such, it is the first receptor in the insulin family to be expressed during development, and its confirmed expression appears to be constitutive in both cell populations of the blastocyst. Since production of IGF-2 mRNA has been observed in embryos of the same stages, concurrent appearance of receptor and ligand suggest auto/paracrine actions by IGF-2 on embryonic development from the time of activation of the embryonic genome. This contrasts with insulin, which does not appear to be expressed by embryos at any preimplantation stage (Rappolee *et al.* 1989*a*) and whose receptor is not expressed until compaction of 8-cell embryos begins (Rappolee *et al.* 1989*a*,*b*; Harvey and Kaye, 1988, 1990).

It will be of interest to compare actions of IGF-2 on embryonic metabolism and development with those of the other members of the insulin family, since insulin receptors appear to regulate mitotic and metabolic effects whilst IGF-1 receptors only mediate mitosis (Harvey and Kaye, 1989).

We thank Dr R. Baxter (Royal Prince Alfred Hospital, Sydney, Aust.) for the generous gift of IGF-2 receptor antibody and Thomas L. Jeatran, of Lilly Research Laboratories, Indianapolis, Indiana USA for the IGF-2, Lindsay Shannon for photography, and Kathryn Markham for technical assistance. This work was supported by a Research Project Grant to P.L.K. and a Biomedical Postgraduate Research Scholarship to M.B.H. from the National Health and Medical Research Council of Australia.

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*(Accepted 3 January 1991)*