Int. J. Dev. Biol. 40: 361-367 (1996)

Expression pattern of different gap junction connexins is related to embryo implantation

RUTH GRÜMMER, BERNHARD REUSS and ELKE WINTERHAGER*

Institute of Anatomy, University of Essen, Essen, Germany

ABSTRACT Successful implantation in mammals requires a close interaction between the embryo and the uterus. Direct cell-cell communication via gap junctions seems to play an important role in the preparation of the uterus for embryo implantation and in the regulation of trophoblast invasion. During preimplantation in the rat the gap junctional proteins connexin (cx) 26 and cx43 are suppressed. This loss of cell-cell communication seems to be important for transformation of the endometrium into the receptive phase. The suppressive effect is mediated by progesterone as demonstrated by the application of antigestagens. At implantation, however, a spatial and temporal pattern of connexin expression is induced in response to embryo recognition. cx26 is locally expressed in the uterine epithelium of the implantation chamber, cx43 in the surrounding decidua prior to invasion. With progressing invasion, the decidual cells surrounding the invading trophoblast in addition to cx43 reveal cx26. In this phase, the invasive partner, the blastocyst, is characterized by coexpression of cx43 and cx31. During trophoblast invasion however, cx31 becomes restricted to the cells of the invasive ectoplacental cone, cx43 to the embryo proper. It seems that compartmentalization of the trophoblast and the inner cell mass is established by two different connexins. During placental differentiation connexin expression switches from cx31 to cx26 and cx43, indicating the end of the invasive phase. The highly regulated pattern of connexin expression in the endometrium as well as in the trophoblast suggests a key role of this different intercellular pathways in regulating the invasion process of the trophoblast into its host tissue, the endometrium.

KEY WORDS: *gap junctions, connexins, trophoblast, endometrium, hormones*

Introduction

In mammals an intimate contact between embryo and mother is needed for successful implantation. Though the modus of implantation is highly divergent among species, all invasive types of implantation share the same cell biological process of trophoblast apposition, adhesion and its penetration into the endometrium (Schlafke and Enders, 1975). The cell biological analysis of this critical period requires the study of independent events in the embryo and the mother and also of interactions between both partners. The endometrial changes involved in the establishment of pregnancy including the pre- and periimplantation phase are governed by steroid hormones (Psychoyos, 1976, 1992). Ovarian steroids change the uterine epithelium from a nonadhesive state to the so-called "receptive phase" which allows the adhesion and invasion of the trophoblast (Schlafke and Enders, 1975; Schlafke *et al.*, 1985; Denker, 1990). In this initial phase the highly invasive trophoblast penetrates into the uterine stroma to arrode the maternal vessels and to establish a hemochorial placenta. This behavior resembles that of malignant tumor cells. However, in contrast to tumor cells trophoblast invasion is strictly controlled by the uterine environment indicating the close interaction of both partners. Local interactions between embryo and endometrium are already described (Dey and Johnson, 1986) and embryonic signals modulating the uterine environment seem to be necessary for initiation and regulation of implantation (for review see Kennedy, 1983).

Intercellular communication via gap junctions, which is involved in cell differentiation, seems to play an important role in preparing the uterus for embryo implantation as well as in the control of trophoblast invasion. Gap junctions are channels which connect the cytoplasms of neighbouring cells and allow ions and small molecules to pass from one cell to another, thereby coupling the cells both electrically and metabolically (reviews: Loewenstein, 1988; Bennett *et al.*, 1991). Each hemichannel is formed by six proteins (connexins) radially arranged around the pore (Fig. 1). About 15 different connexins, which all belong to a multigene family, have already been cloned (Beyer *et al.*, 1990; Willecke *et al.*, 1991; Haefliger *et al.*, 1992) and their sequences show very high identity between the species investigated. Although different unit conductances for gap junction channels comprised of different connexins have been recorded (rev: Kolb

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Abbreviations used in this paper: dpc, days post coitum; cx, connexin.

*Address for reprints: Institut für Anatomie, Universitätsklinikum Essen, Hufelandstr. 55, D-45122 Essen, Germany. FAX: 49.201-723 5916.

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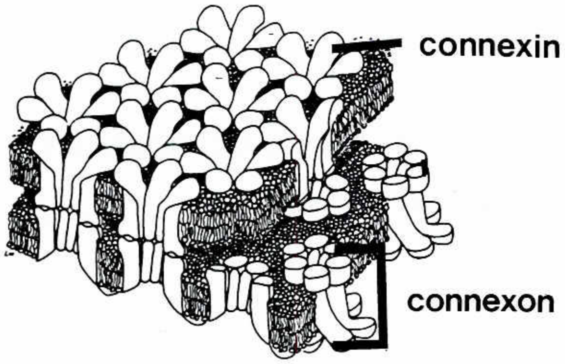

Fig. 1. Schematic drawing of gap junctional channels. *Each hemichannel (connexon) is composed of six protein subunits (connexins) (Makowski et al., 1977).*

and Somogyi, 1991; Ramanan *et al.*, 1993), the physiological role of those channels still remains unknown. Thus the expression pattern of various connexin genes may be related to different stages of cellular differentiation and function.

In the recent years we have focused our interest on gap junction expression in both compartments, in the developing embryo as well as in the endometrium, during the pre- and periimplantation phase as a cell biological marker for their differentiation during trophoblast adhesion and invasion into the maternal tissue.

Connexin expression in the endometrium during pre- and periimplantation

Suppression of cell-cell communication during preimplantation

In the rat endometrium only two gap junctional connexins, cx26 and cx43, seem to play a role during the cyclic phases of nonpregnancy and during early pregnancy. In all cyclic phases of nonpregnancy cx26 as well as cx43 mRNA is evidenced in high amounts whereas the corresponding protein is rarely found (Winterhager *et al.*, 1993). During endometrial transformation to the "receptive phase" both connexin transcripts are completely suppressed in the endometrium of the preimplantation phase (Fig. 2a,b) (Grümmer *et al.*, 1994). This suppression of cx26 and cx43 and thereby the loss of direct cell-cell communication is under control of maternal progesterone. Applying the antiprogestin onapristone (ZK 98 299) on the first 2 or 3 days of pregnancy, a withdrawal of the connexin suppression can be observed (Fig. 2a), showing cx26 expression in the uterine epithelium (Fig. 2c) and a cx43 staining in the stromal cells in the preimplantation phase (Grümmer *et al.*, 1994). The influence of maternal steroid hormones on connexin expression in the endometrium could also be evidenced in ovariectomized rats treated with 17-ß-estradiol and/or progesterone. Untreated rats show cx43 mRNA, but no transcript for cx26. Estradiol drastically increases levels of both cx26- and cx43-mRNA whereas application of progesterone leads to a clear decrease of connexin transcripts. When estradiol and progesterone are administered simultaneously, the suppressive effect of progesterone predominates and no connexin expression can be found. Thus connexin expression in rat endometrium is regulated by maternal steroid hormones: Cx26 and cx43 are suppressed by progesterone and enhanced by estradiol. The suppressive effect of progesterone leads to a noncoupled endometrium during early pregnancy. Since nearly all tissues exhibit cell-cell communication, it is a phenomenon that the uterine epithelium is noncoupled during the preimplantation phase. This seems to be very important in regard to differentiation to a receptive epithelium as application of antiprogestins during this first days of pregnancy inhibit embryo implantation in rats and guinea pigs (Elger *et al.*, 1986; Roblero and Croxatto, 1991).

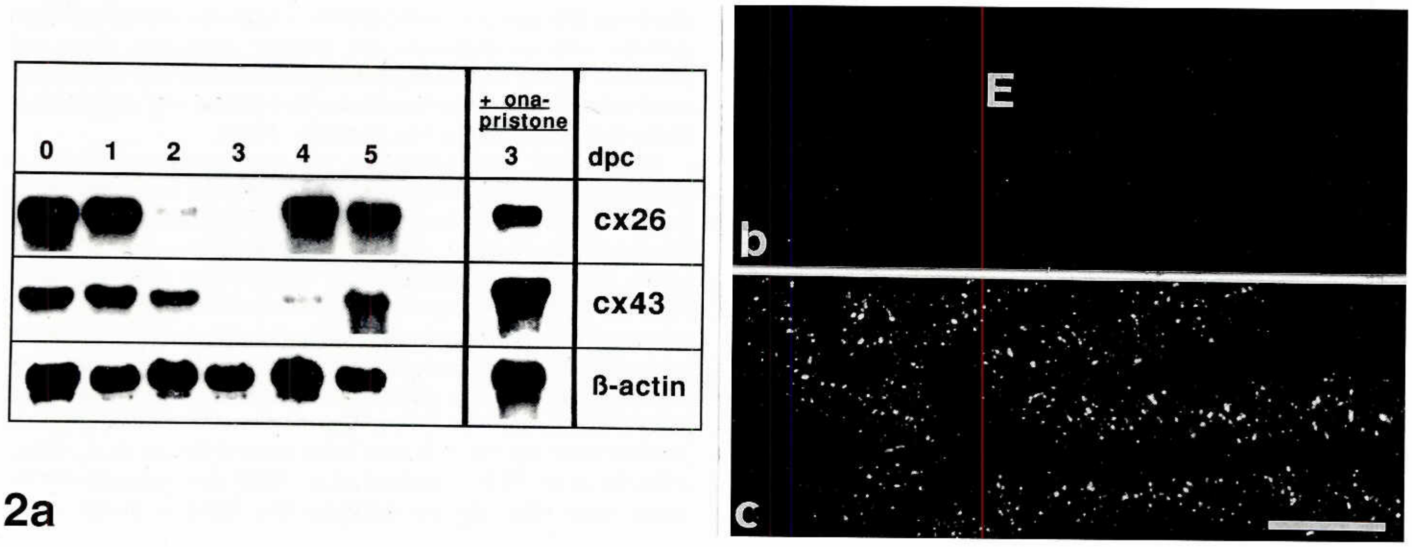

Fig. 2. Connexin expression in the rat endometrium during the periimplantation phase. (a) *Northern blot analysis of rat endometrial RNA from 0-5 dpc probed for cx26 and cx43 mRNA. In the preimplantation period (1-3 dpc) levels of mRNA for both connexins decline. At implantation (4 dpc) mRNA levels increase for both connexins. Onapristone treatment of pregnant rats on 0-2 dpc inhibited the decrease in connexin-mRNA levels observed on 3 dpc in untreated rats.* **(b,c)** *Immunohistochemical staining of cx26 in the rat endometrium on day 3 pc.* **(b)** *No staining of cx26 can be observed in the uterine epithelium (E).* **(c)** *Rats treated with onapristone on 0-2 dpc show a strong staining for cx26 in the luminal epithelial cells. Bars, 70 µm.*