BRIEF COMMUNICATION

Expression of Connexin 30 in *Xenopus* Embryos and Its Involvement in Hatching Gland Function

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**ABSTRACT** Connexins are a family of proteins that assemble to form gap junction channels. Cell-cell communication through gap junctions mediates many important events in embryogenesis, including limb patterning, lens physiology, neuronal function, left-right asymmetry, and secretion from gland tissue. We studied the expression of connexin 30 (Cx30) in the *Xenopus* embryo and find that it is expressed in the developing hatching gland and pronephros. To determine whether its expression plays a functional role in the activity of the hatching gland, we exposed pre-hatching embryos to drugs that block gap junctional communication. This resulted in a continuation of normal growth and development but specifically abolished hatching. The treatment did not affect Cx30 or *Xenopus* hatching enzyme transcription, suggesting a post-transcriptional effect on Cx30 gap junctions. We conclude that junctional communication, possibly mediated by Cx30, is involved in secretion of hatching enzyme in *Xenopus*. Dev Dyn 2000;219:96–101. © 2000 Wiley-Liss, Inc.

Key words: connexins; gap junctions; hatching; secretion; *Xenopus*

**RESULTS AND DISCUSSION**

We examined the expression pattern of connexin 30 (Gimlich et al., 1988) in *Xenopus* embryos by whole-mount in situ hybridization. *Xenopus* connexin 30 is most similar to mouse connexin 32 (58.7% identity). We first detect expression of Cx30 or *Xenopus* hatching enzyme transcription, suggesting a post-transcriptional effect on Cx30 gap junctions. We conclude that junctional communication, possibly mediated by Cx30, is involved in secretion of hatching enzyme in *Xenopus*. Dev Dyn 2000;219:96–101. © 2000 Wiley-Liss, Inc.

INTRODUCTION

Gap junctional channels formed by oligomers of proteins from the connexin family are pores between cells that allow the conduction of low-molecular-weight molecules (< 1 kd) (Bruzzone et al., 1996; Goodenough et al., 1996). Gap-junctional communication (GJC) of important regulatory signals underlies many important physiological phenomena, such as left-right asymmetry (Levin and Mercola, 1998; Levin and Mercola, 1999), carcinogenesis (Yamasaki et al., 1995; Krutovskikh and Yamasaki, 1997), and neuronal function (Bruzzone and Ressot, 1997; Dermietzel, 1998). GJC is also an obligatory feature of most gland tissues, playing a role in regulating key secretory events (Meda, 1996a, 1996b). For example, in the mammalian pancreas, abolishing GJC renders cells unable to secrete insulin (Meda et al., 1990). Despite the importance of gap junctions in many physiological events, few studies have examined the spatial patterns of expression of connexin proteins in early chick or frog embryos. In this study we characterized the expression of *Xenopus* Cx30 in embryogenesis and examined the possible role of GJC in the function of the hatching gland.

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This finding may indicate divergent function of connexin family members in different species.

In order to investigate the hatching gland expression more closely, we compared Cx30 staining to the pattern of expression of the gene encoding the *Xenopus* hatching enzyme (Katagiri et al., 1997). *XHE* encodes a metalloprotease secreted by hatching gland cells to allow the embryo to escape from the vitelline membrane and is expressed in the ectoderm on the anterior dorsal portion of the head and face (Fig. 2A,B). Similarly, Cx30 is expressed in identical locations during...
We conclude that Cx30 is expressed in tissue that secretes XHE. Cx30 is a marker specific to the hatching gland (see also Drysdale and Elinson [1991]), in contrast to other available markers of the hatching gland, XA-1 (Hemmatti-Brivanlou et al., 1990; Sive and Bradley, 1996) and XAG (Sive et al., 1989), which stain both the hatching gland and the cement gland.

We then tested the functional role of GJC in the activity of the hatching gland by inhibiting the function of endogenous gap junctions. This was done by exposing pre-hatching embryos to drugs (heptanol, glycyrrhetinic acid, and anandamide) that have been shown to close gap junctions rapidly in mammalian and Xenopus systems and by targeting injections of a dominant-negative connexin (H7) to the hatching gland (Davidson and Baumgarten, 1988; Chanson et al., 1989; Takens-Kwak et al., 1992; Venance et al., 1995; Levin and Mercola, 1998). In previous studies, we have shown that injection of H7 and exposure to several GJC drugs rapidly (< 1.5 hr) decrease GJC in Xenopus embryos (Levin and Mercola, 1998).
Figure 3.

Figure 4.
Thus, we monitored hatching in batches of embryos whose medium contained heptanol, glycyrrhetinic acid, or anandamide. These batches exhibited far fewer hatched embryos when examined at a time point during the hatching process. Control embryos were all hatched from the vitelline membrane by stage 29 (Fig. 3A). In contrast, embryos exposed from stage 22 to drugs that inhibit the action of connexins were unable to hatch at the normal time (Fig. 3B). Only 52% of embryos (n = 27) exposed to glycyrrhetinic acid were hatched at a point when 100% of the control embryos (n = 30) had escaped the vitelline membrane (χ^2 = 16.08; P = 6 × 10^{-6}). A similar result was observed with anandamide and heptanol (two other GJC-reducing drugs; data not shown). By stage 37, control embryos are always hatched and develop normally (Fig. 3D), and by these stages most of the embryos exposed to glycyrrhetinic acid have also hatched. Prolonged exposure to anandamide, however, caused half the embryos (n = 26) to remain trapped in the vitelline membrane as late as stage 41 (Fig. 2E,F). Similar results were observed using heptanol (data not shown).

We also microinjected mRNA encoding a hybrid connexin construct, H7, which acts as a dominant negative to block GJC. Embryos injected with H7, which showed targeting to the hatching gland, also failed to hatch (Fig. 3C). A similar hatchling phenotype has been described (Elinson, 1974) in embryos resulting from cross-species fertilization in frogs. However, based on the specific construct injections as well as the drug exposure data, we conclude that functional gap junctions are required for hatching gland function and that Cx30 is a likely candidate for this role. We cannot rule out the presence and involvement of other connexins in the hatching gland, because known pharmacological and dominant-negative inhibitors of GJC affect multiple members of the connexin family.

In order to determine whether the effect of GJC-reducing drugs on the function of the hatching gland occurs at the mRNA or protein level, we examined the expression of the Cx30 and XHE genes in embryos treated with glycyrrhetinic acid. Embryos were exposed to glycyrrhetinic acid at stage 18 and fixed at stages 24–29. Compared with control embryos (Fig. 4A,C), no differences in the expression pattern of Cx30 (Fig. 4B; n = 25) and XHE (Fig. 4D; n = 27) were detected in embryos whose GJC was inhibited by glycyrrhetinic acid. We conclude that, in agreement with models for GJC function in other gland tissue, inhibition of hatching enzyme secretion by GJC-reducing agents occurs at the level of connexin protein regulation, not through down-regulation of connexin mRNA expression.

GJC is known to be involved in embryonic muscle development (Armstrong et al., 1983; Mege et al., 1994; Todman et al., 1999). However, the hatching defect is unlikely to be due to the drugs’ inhibition of muscle activity, because embryos cultured in these drugs are able to move normally and because embryos cultured in tricaine, which paralyzes the embryos, are still able to hatch (data not shown).

In glands such as the pancreas and thyroid, GJC is thought to play a role in secretions by synchronizing Ca^2+ oscillations and equalizing voltage between groups of cells (Meda, 1996a; Bertuzzi et al., 1999; Hofer, 1999). Significant GJC between groups of cells results in a syncytium and allows diffusion of small molecules through a tissue. This can result in the equalization of ionic and molecular gradients and ensures spread of signals controlling secretion. We suggest that inhibition of cell-cell communication disrupts the hatching process by interfering with hatching gland function. Our study of the expression of Cx30 suggests that it as a likely candidate to mediate GJC in the hatching gland. Expression of Cx30 in the lining of the archereteron and in pronephros suggests possible other roles for this gap junction protein in several organ systems in Xenopus embryogenesis.

**EXPERIMENTAL PROCEDURES**

In Situ Hybridization

In situ hybridization was performed as previously described. Antisense probe labeled with digoxigenin was generated from the Cx30 (Gimlich et al., 1988) and XHE (Katagiri et al., 1997) clones.

Drug Exposure

Embryos were transferred to 0.1× MMR medium containing anandamide, 18α-glycyrrhetinic acid, or heptanol (prepared as described by Levin and Mercola, 1998) at stage 22.

Dominant-Negative Construct Injection

Synthetic mRNA was transcribed by the SP6 polymerase from linearized SP64T plasmids containing the individual cDNAs. About 50 pg of H7 mRNA was mixed with 50 pg of RLD and 250 pg of mRNA encoding β-galactosidase (as lineage labels) and injected into the very top of the animal pole of both cells in two-cell-stage embryos.

Statistical Analysis

Significance of numerical data was computed by the χ-square test with Pearson correction (a more stringent version of the χ-square test).

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