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Roles of Na,K-ATPase in early development and trophectoderm differentiation.

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Before implantation into the uterine wall, the mammalian embryo undergoes a period of cell division, cell shape change, and cell differentiation leading to the formation of an outer epithelium, the trophectoderm. The trophectoderm is the part of the embryo that initiates uterine contact and, after transformation to become the trophoblast, uterine invasion. Similar to the kidney nephron, the trophectoderm is a transporting epithelium with distinct apical and basolateral membrane domains; its function is to facilitate transepithelial Na^+ and fluid transport for blastocoel formation. That transport is driven by Na,K-adenosine triphosphatase (ATPase) localized in basolateral membranes of the trophectoderm. Preimplantation embryos express multiple α and β subunit isoforms of Na,K-ATPase, potentially constituting multiple isozymes, but the basolaterally located $\alpha 1\beta 1$ isozyme appears to function uniquely to drive fluid transport. Embryos unable to express $\alpha 1$ subunits because of targeted deletion of the gene are able to form a blastocoel, but they fail to maintain their integrity and expire during the peri-implantation period. Preimplantation embryos also express the γ subunit, a modulator of Na,K-ATPase activity, but targeted deletion of that gene did not reveal an essential developmental role. The preimplantation embryo offers a unique model for understanding the roles of Na,K-ATPase subunit isoforms in epithelial development and transepithelial transport.
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In addition to its well-studied roles in membrane potential and membrane transport processes, Na,K-adenosine triphosphatase (ATPase) is thought to play an essential role in the earliest phase of mammalian embryogenesis, that which precedes implantation of the embryo into the uterus. Preimplantation development is a period of cell division, cell shape change, and cell differentiation leading to the formation of a polarized, transporting epithelium, the trophectoderm (reviewed by Watson and Barcroft[1]). The trophectoderm forms the outer wall of the blastocyst, the stage of development that immediately precedes implantation. It is the part of the conceptus that initiates uterine contact and, after transformation to become the trophoblast, uterine invasion. Thus, trophectoderm development during preimplantation stages is a necessary antecedent to the events of implantation.

Although common to all eutherians, the processes involved in trophectoderm development have been studied most thoroughly in the mouse. After 3 cleavage divisions the mouse embryo undergoes a process of compaction in which blastomeres flatten against one another, polarize, and begin to assemble adherens, gap, and tight junctions (reviewed by Fleming et al[2]). Within the next 2 cell cycles the outer cells of the embryo become specialized as an epithelial monolayer. It is the ion- and fluid-transporting ability of this epithelial trophectoderm, driven by Na,K-ATPase, that enables blastocoel formation (cavitation). The blastocoelic fluid provides a unique extracellular environment for the inner cell mass, which eventually will give rise to the fetus. In addition to its roles in establishment of pregnancy, trophectoderm arises de novo from previously unspecialized blastomeres, making it a unique model for understanding how a polarized transporting epithelium develops.[3,4]

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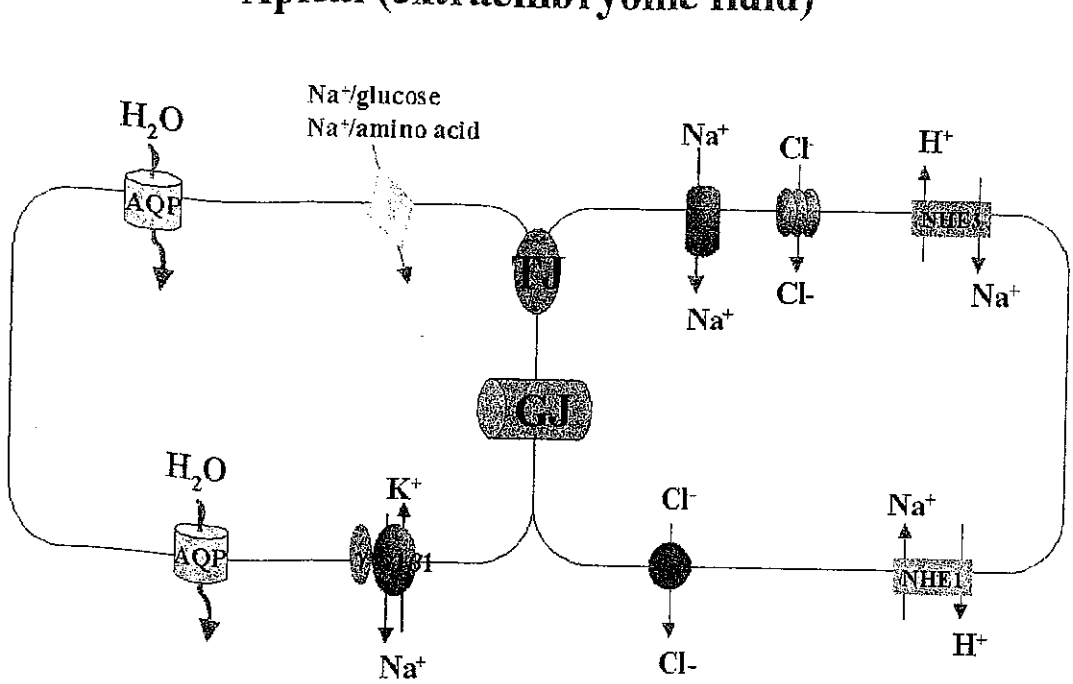

Figure 1 Arrangement of transport systems known to be involved in transepithelial Na^+ transport in mouse trophectoderm. Two trophectoderm cells are shown, joined by a tight junction (TJ) and a gap junction (GJ). Basolateral sodium pumps (Na,K-ATPase) work in conjunction with apical routes of Na^+ entry that include the NHE-3 isoform of the Na^+/H^+ exchanger (NHE), the amiloride-sensitive epithelial sodium channel, and Na^+-linked glucose and amino acid transporters. Water crosses the trophectoderm via aquaporin channels in both apical and basolateral membranes. Vectorial transport of Na^+ and water across the epithelial trophectoderm is hypothesized to cause the blastocoel cavity to expand before implantation. Although other Na,K-ATPase subunit isoforms are present in trophectoderm cells, the $\alpha 1$ and $\beta 1$ subunits are the only ones confined to basolateral membrane domains where they colocalize with γ subunits. Adapted from Watson and Barcroft[1]. (Color version of figure is available online.)

The Role of the Sodium Pump in Blastocoel Formation

Na,K-ATPase activity can be shown in all stages of preimplantation development,[5,6] but it seemingly plays a specific morphogenetic role at the time of cavitation.[1] Expansion of the mammalian blastocoel is caused by transport of fluid across the trophectoderm, and this process can be prevented by ouabain, a specific inhibitor of Na,K-ATPase.[7-10] The involvement of Na,K-ATPase also is supported by the fact that blastocoel expansion is retarded significantly in the absence of extraembryonic Na^+ or in the presence of inhibitors of Na^+ channels or carriers with access to the apical trophectoderm surface.[8] Clarification of the way the enzyme works in this context was provided by immunolocalization experiments showing that it is concentrated in the basolateral plasma membranes of the trophectoderm.[11-13] Treatments that disrupt or prevent the development of the membrane-cytoskeletal complex in the blastocyst also prevent Na,K-ATPase from assuming its basolateral localization, and fluid transport is blocked.[3,4] The model on which these experiments were focused, shown in Fig. 1, is that the basolateral localization of Na,K-ATPase allows polarized pumping of Na^+ across the trophectoderm, setting up an osmotic gradient to cause fluid to accumulate in the blastocoel. Several apical routes of Na^+ entry into trophectoderm cells have been identified that would work in conjunction with basolateral sodium pumps to provide a transtrophectodermal Na^+ flux.[8,14,15] Furthermore, several aquaporin family members have been identified in apical and basolateral trophectoderm membranes and evidence was presented that these aqueous channels facilitate the rapid movement of water into the blastocoel under near–iso-osmotic conditions.[16,17]

Sodium Pump α and β Subunit Isoforms in Preimplantation Embryos

Based on the co-expression of multiple α and β subunit isoforms in preimplantation embryos of both mouse and cow, multiple (perhaps as many as 6) Na,K-ATPase isozymes could be present, adding complexity to our understanding of the roles that this enzyme plays in trophectoderm develop-

Table 1 Expression of Na,K-ATPase Subunit Isoforms in Mouse Blastocysts

Method	α1	α2	α3	α4	β1	β2	β3
RT-PCR	+	−	+	−	+	+	+
Western blot	+	−	+	ND	+	+	+
Immunofluorescence	Basolateral		Cytoplasmic	ND	Basolateral	Cytoplasmic	Cytoplasmic

RT-PCR, reverse-transcription polymerase chain reaction; ND, not determined.
Data from MacPhee et al.[13]

ment and function (see Table 1).[10,13] However, confocal immunofluorescence microscopy has revealed only α1 and β1 subunits in basolateral trophectoderm membranes, indicating that the α1β1 isozyme is involved uniquely in active transport of Na^+ and water into the blastocoel.[13,18] Interestingly, in the cow (but not the mouse), α3 subunits are present predominantly in apical membranes of the trophectoderm[18]; whether this subunit isoform has a specific role to play in the maximally expanding cow blastocyst remains to be determined. In both species, blastocoel formation is correlated temporally with up-regulation of expression of β1 subunits, suggesting that it may be triggered by that event.[9,10,13,19]

In the mouse, specific functions for individual sodium pump subunit isoforms have been explored by targeted disruption of the encoding genes. For example, an essential role for α2 and β2 subunits in preimplantation development has been ruled out by showing that mice lacking either of these subunits are born alive at full term.[20,21] Absence of the α1 subunit, on the other hand, developmentally is lethal.[21] Heterozygous mice that express only 1 copy of the Na,K-ATPase α1 subunit gene are fertile and generally are healthy, but homozygous null offspring were not found among their progeny. Based on earlier studies (cited earlier), it was hypothesized that an active α1β1 isozyme would be required to mediate blastocoel formation and that the absence of α1 null mutant offspring therefore must reflect failure of the mutant embryos to reach the blastocyst stage and achieve competence to implant. Surprisingly, when development of the mutant embryos was followed-up in vitro, it was found that they can develop to the blastocyst stage in normal numbers and are indistinguishable morphologically from their wild-type counterparts.[22] Eventually, however, the mutant blastocysts dissociated, losing trophectodermal integrity, and failed to escape from the zona pellucida, the extracellular matrix that surrounds the developing embryo. Because escape from the zona in vitro is known to result from the activity of a proteolytic enzyme secreted by the trophectoderm,[23] this observation indicates that the health of the trophectoderm had been compromised in the absence of α1 subunits. The α1 null mutant blastocysts also were incapable of forming outgrowths in vitro, a process that mimics some aspects of implantation.[22] These observations indicate that although the survival of α1 null mutant embryos is short-lived, they are able to progress to the blastocyst stage but die shortly after, during peri-implantation development. It remains to be determined whether expression of any of the other α subunit isoforms is altered in α1 null mutant embryos to maintain sodium pump activity, allowing the blastocoel to form.

The γ Subunit

The γ subunit is a small type I membrane protein, a member of the FXYD family, that modulates the activity of the sodium pump in specific cell types.[24-26] It is most abundant in the kidney, where it is highly expressed in certain distal nephron segments.[27-30] Despite the fact that the γ subunit, unlike the α and β subunits, is encoded by a single gene (designated *FXYD2* by Sweadner and Rael[26]), there are 2 γ subunit isoforms in kidney with different N-terminal amino acid sequences, most likely arising from alternate splicing.[26,31,32] With the cloning of the mouse *Fxyd2* gene it became apparent that there actually are 3 variants in that species, also differing in their N-termini.[33] Each of the 3 N-termini links with the common transmembrane domain.

Given the functional similarities between the blastocyst trophectoderm and the kidney nephron, it was of interest to explore the possibility that γ subunits also play a role in preimplantation development. The γ subunit gene is transcribed continuously in the mouse preimplantation embryo from the 8-cell stage onward and γ subunits accumulate and localize to the peripheries of blastomeres as development proceeds.[34] While colocalizing with the α1β1 isozyme in the basolateral membranes of the trophectoderm, γ subunits also appear to be expressed in the apical membranes where α and β subunits are not detectable by immunofluorescence (Fig. 1).[13,34,35] Messenger RNAs encoding both γa and γb variants are present in blastocysts.[33] Mice were generated that lacked the common transmembrane-encoding sequence of the *Fxyd2* gene, a deletion that would be expected to abolish the function of all 3 γ isoforms. Surprisingly, mice homozygous for this deletion were viable and fertile and without obvious pathology (Jones et al,[36]). The absence of any effect on blastocoel formation was confirmed by showing no correlation between the timing of blastocyst development and embryo genotype resulting from heterozygote crosses. The possibility that null mutant embryos were being rescued by γ subunits contributed by the oocyte was ruled out by the fact that expected Mendelian ratios of offspring were obtained even from $Fxyd2^{-/-}$ dams. Thus, γ subunits lack an essential role in preimplantation development.

Summary

Despite the expression of multiple members of each of the Na,K-ATPase subunit gene families during preimplantation development, and determination of the role of the enzyme in supporting blastocoel formation by studies using pharmaco-

logic inhibitors, we still have not defined the individual role of each expressed isoform. Thus far, the α1 isoform is the only one determined to play an essential role in preimplantation development. Research directed at understanding the role of Na,K-ATPase isozymes during embryogenesis will continue well into the future.

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