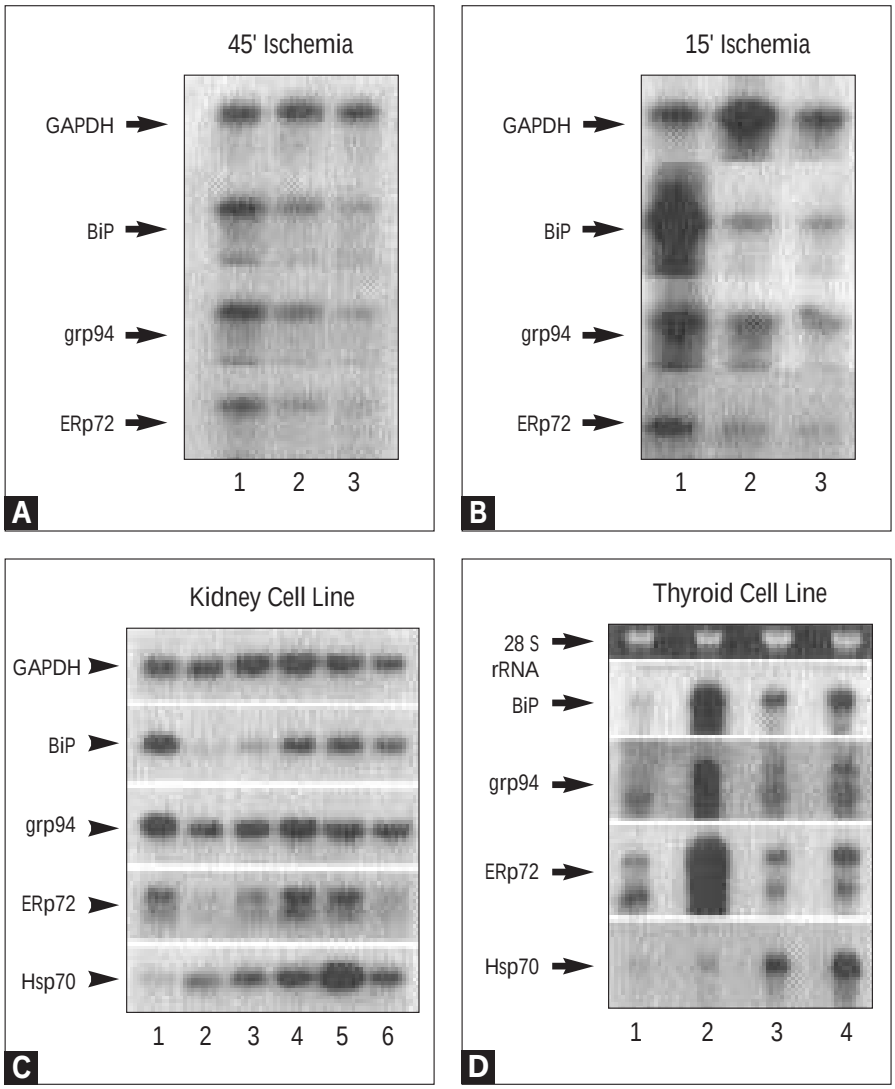

FIGURE 16-11

Ischemia upregulates endoplasmic reticulum (ER) molecular chaperones. Molecular chaperones of the ER are believed to function normally to prevent inappropriate intra- or intermolecular interactions during the folding and assembly of proteins [20, 54]. However, ER molecular chaperones are also part of the "quality control" apparatus involved in the recognition, retention, and degradation of proteins that fail to fold or assemble properly as they transit the ER [20, 54]. In fact, the messages encoding the ER molecular chaperones are known to increase in response to intraorganelle accumulation of such malfolded proteins [11, 20, 54, 55]. Here, Northern blot analysis of total RNA from either whole kidney or cultured epithelial cells demonstrates that ischemia or ATP depletion induces the mRNAs that encode the ER molecular chaperones, including immunoglobulin binding protein (BiP), 94 kDa glucose regulated protein (grp94), and 72 kDa endoplasmic reticulum protein (Erp72) [11]. This suggests not only that ischemia or ATP depletion causes the accumulation of malfolded proteins in the ER but that a major effect of ischemia and ATP depletion could be perturbation of the "folding environment" of the ER and disruption of protein processing. GAPDH—glyceraldehyde-3-phosphate dehydrogenase; Hsp70—70 kDa heat-shock protein. (*From* Kuznetsov *et al.* [11]; with permission.)

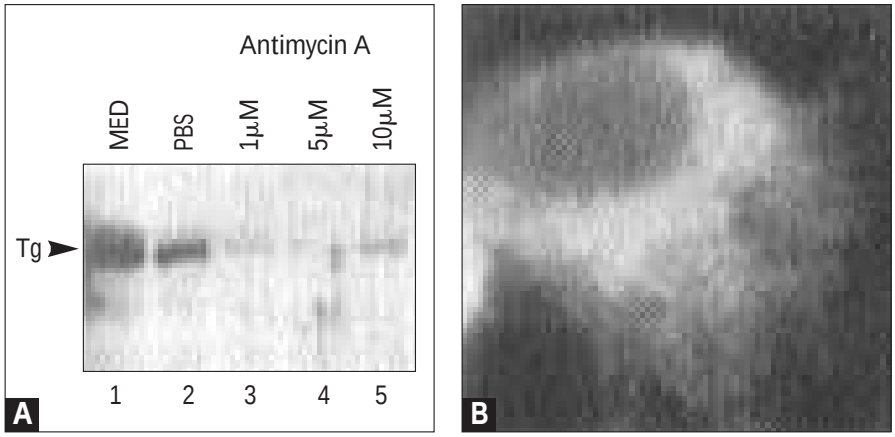

FIGURE 16-12

ATP depletion perturbs normal endoplasmic reticulum (ER) function. Because ATP and a proper redox environment are necessary for folding and assembly [20, 54, 63, 64] and ATP depletion alters ATP levels and the redox environment, the secretion of proteins is perturbed under these conditions. Here, Western blot analysis of the culture media from thyroid epithelial cells subjected to ATP depletion (*ie*, treatment with antimycin A, an inhibitor of oxidative phosphorylation) illustrates this point. **A**, Treatment with as little as 1μM antimycin A for 1 hour completely blocks the secretion of thyroglobulin (Tg) from these cells.

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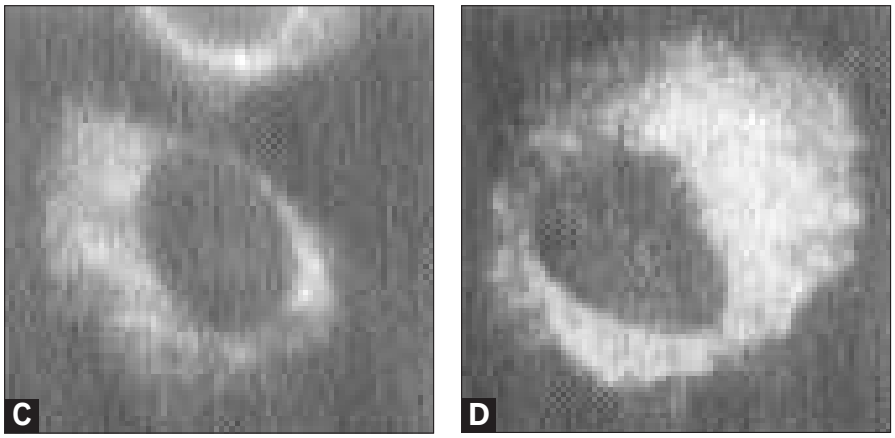


FIGURE 16-12 *(Continued)*

B–D, Moreover, indirect immunofluorescence with antithyroglobulin antibody demonstrates that the nonsecreted protein is trapped almost entirely in the ER. Together with data from Northern blot analysis, this suggests that perturbation of ER function and disruption of the secretory pathway is likely to be a key cellular lesion in ischemia [11]. MED—control media; PBS—phosphate-buffered saline. (*From* Kuznetsov *et al*. [11]; with permission.)

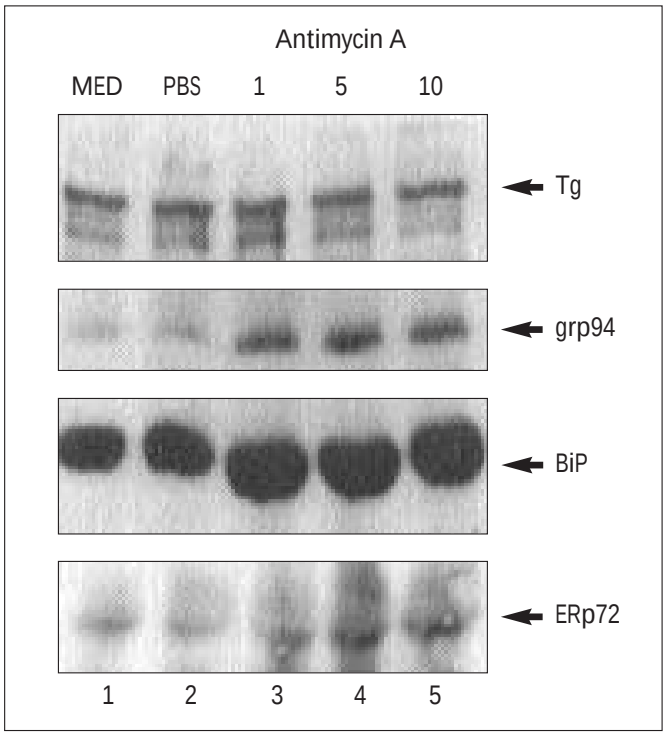
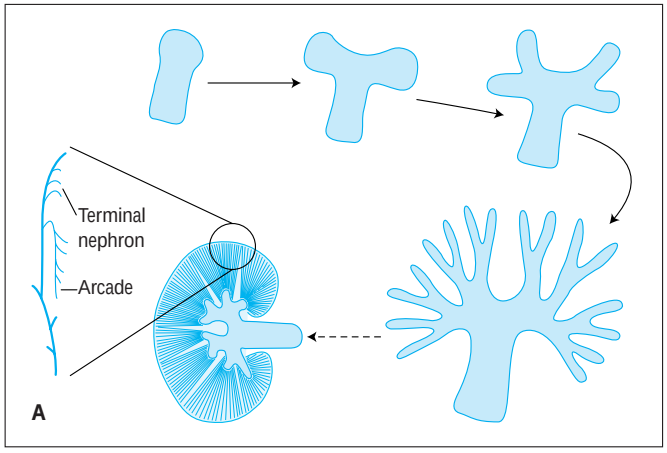
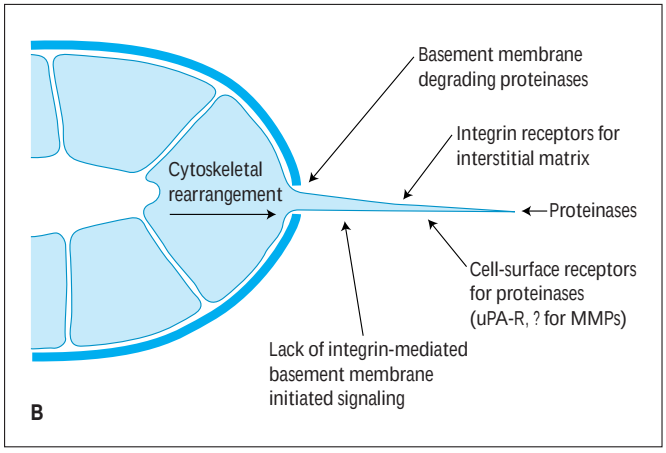


FIGURE 16-13

ATP depletion increases the stability of chaperone-folding polypeptide interactions in the endoplasmic reticulum (ER). Immunoglobulin binding protein (BiP), and perhaps other ER molecular chaperones, associate with nascent polypeptides as they are folded and assembled in ER [20, 54, 56, 57, 65–73]. The dissociation of these proteins requires hydrolysis of ATP [69]. Thus, when levels of ATP drop, BiP should not dissociate from the secretory proteins and the normally transient interaction should become more stable. Here, the associations of ER molecular chaperones with a model ER secretory protein is examined by Western blot analysis of thyroglobulin (Tg) immunoprecipitates from thyroid cells subjected to ATP depletion. After treatment with antimycin A, there is an increase in the amounts of ER molecular chaperones (BiP, grp94 and ERP72) which co-immunoprecipitate with antithyroglobulin antibody [11], suggesting that ATP depletion causes stabilization of the interactions between molecular chaperones and secretory proteins folded and assembled in the ER. Moreover, because a number of proteins critical to the proper functioning of polarized epithelial cells (*ie*, occludin, E-cadherin, Na-K-ATPase) are folded and assembled in the ER, this suggests that recovery from ischemic injury is likely to depend, at least in part, on the ability of the cell to rescue the protein-folding and -assembly apparatus of the ER. Control media (MED) and phosphate buffered saline (PBS)—no ATP depletion; 1, 5, 10μM antimycin A—ATP-depleting conditions. (*From* Kuznetsov *et al*. [11]; with permission.)

Growth Factors and Morphogenesis





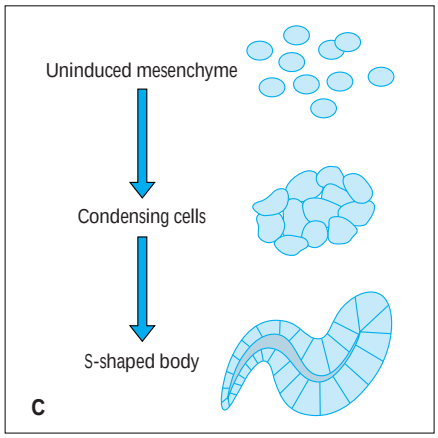


FIGURE 16-14

Kidney morphogenesis. Schematics demonstrate the development of the ureteric bud and metanephric mesenchyme during kidney organogenesis. During embryogenesis, mutual inductive events between the metanephric mesenchyme and the ureteric bud give rise to primordial structures that differentiate and fuse to form functional nephrons [74-76]. Although the process has been described morphologically, the nature and identity of molecules involved in the signaling and regulation of these events remain unclear. **A**, Diagram of branching tubulogenesis of the ureteric bud during kidney organogenesis. The ureteric bud is induced by the metanephric mesenchyme to branch and elongate to form the urinary collecting system [74-76]. **B**, Model of cellular events involved in ureteric bud branching. To branch and elongate, the ureteric bud must digest its way through its own basement membrane, a highly complicated complex of extracellular matrix proteins. It is believed that this is accomplished by cellular projections, "invadopodia," which allow for localized sites of proteolytic activity at their tips [77-81]. **C**, Mesenchymal cell compaction. The metanephric mesenchyme not only induces ureteric bud branching but is also induced by the ureteric bud to epithelialize and differentiate into the proximal through distal tubule [74–76]. (*From* Stuart and Nigam [80] and Stuart *et al*. [81]; with permission.)

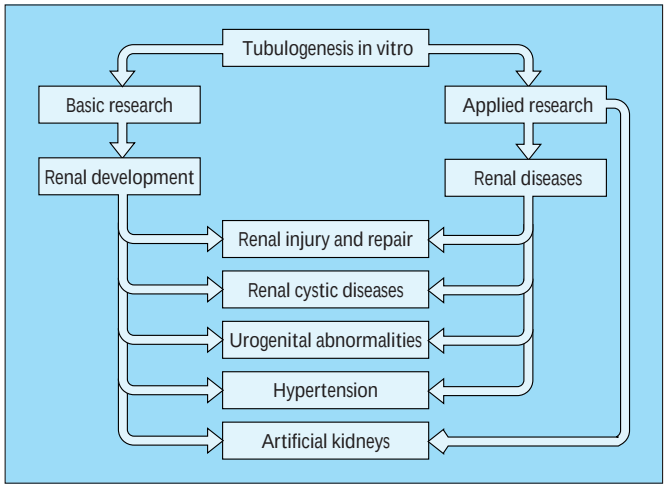


FIGURE 16-15

Potential of in vitro tubulogenesis research. Flow chart indicates relevance of in vitro models of kidney epithelial cell branching tubulogenesis to basic and applied areas of kidney research. While results from such studies provide critical insight into kidney development, this model system might also contribute to the elucidation of mechanisms involved in kidney injury and repair for a number of diseases, including tubular epithelial cell regeneration secondary to acute renal failure. Moreover, these models of branching tubulogenesis could lead to therapies that utilize tubular engineering as artificial renal replacement therapy [82].