Interaction of bcl-2 with Paxillin through Its BH4 Domain Is Important during Ureteric Bud Branching*

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Christine M. Sorenson‡
From the Department of Pediatrics, University of Wisconsin, Madison, Wisconsin 53792

bcl-2 protects cells from apoptosis initiated by a variety of stimuli including loss of cell adhesion. Mice deficient in bcl-2 (bcl-2−/−) develop renal hypoplastic/cystic dysplasia, a condition that leads to significant morbidity and mortality in children. The precise mechanism of action of bcl-2 has not been elucidated. bcl-2 may merely facilitate survival of precursor cells and/or may play a more “active” role during morphogenesis by interacting with other proteins such as paxillin. Recent work in this laboratory demonstrated that bcl-2 directly associates with paxillin. The data presented here demonstrate that the bcl-2 homology 4 (BH4) domain, specifically amino acids 17–31, is necessary for the bcl-2 interaction with paxillin. Paxillin also associated with the BH4 domains of more closely related bcl-2 family members, bcl-xL and bcl-w, compared with that from the non-mammalian homologue ced9. Tyrosines 21 and 28 in the bcl-2 BH4 domain were essential for interaction with paxillin. In embryonic kidney organ culture, incubation with the bcl-2 BH4 domain resulted in inhibition of ureteric bud branching. Therefore, these data suggest that the interaction of bcl-2 with paxillin plays an important role during nephrogenesis.

bcl-2 is a novel proto-oncogene that inhibits apoptosis rather than promoting proliferation. Overexpression of bcl-2 increases the viability of cells under various adverse circumstances including cytokine withdrawal, loss of cell adhesion, and γ-irradiation. bcl-2 is inserted into the outer mitochondrial, endoplasmic reticulum, and nuclear membranes in an Ncyto-Cin orientation with the bulk of the protein facing the cytosol (1). bcl-2 contains four conserved domains denoted bcl-2 homology (BH)1 (residues 136–155), BH2 (residues 187–202), BH3 (residues 93–107), and BH4 (residues 10–30) as well as a transmembrane-spanning region at the carboxyl end of the protein (2). Through the BH domains bcl-2 forms homo- and heterodimers with various bcl-2 family members impacting the regulation of apoptosis.

The BH4 domain is conserved in bcl-2 family members with death-repressing activity: bcl-2 with the BH4 domain deleted (amino acids 10–30) lacks the survival activity (3). Removal of the amino-terminal portion of bcl-2 by cleavage by caspase 3 or trypsin-like protease results in a carboxyl fragment that can accelerate cell death (4, 5). The BH4 domain interacts with non-family members including Raf-1 kinase, calcineurin, and ced4 (3, 6, 7). Unfortunately, the mechanism by which bcl-2 imparts its death-inhibitory activity and whether bcl-2 functions in other capacities require further investigation.

bcl-2 expression can be regulated by interaction of integrins with components of the basement membrane. For example, overexpression of bcl-2 in epithelial cells promotes dedifferentiation, a loss of expression of E-cadherin and α2β1 integrin, and multilayer formation at high density (8). bcl-2 transcription is elevated in cells attached to fibronectin through α5β1 or vitronectin through αvβ3 (9). Regulation of cell survival function by bcl-2 requires FAK, Shc, and activated Ras (through phosphatidylinositol 3-kinase/Akt pathway) (9).

Recent work in this laboratory demonstrated that bcl-2 co-immunoprecipitated with FAK and paxillin in embryonic kidney lysates (10). Utilizing Far Western analysis, the interaction between bcl-2 and paxillin was shown to be direct, whereas its interaction with FAK was indirect. Gaining a better understanding of the molecular and cellular pathways by which bcl-2 mediates cell survival is essential for determining its role during development. Here the association between bcl-2 and paxillin was further examined, demonstrating that the BH4 domain, specifically amino acids 17–31, is necessary for the bcl-2 interaction with paxillin. Paxillin also associated with the BH4 domains of more closely related bcl-2 family members, bcl-xL and bcl-w, compared with that from the non-mammalian homologue ced9. Tyrosines 21 and 28 in murine bcl-2 were essential for interaction with paxillin. In embryonic kidney organ culture, incubation with the bcl-2 BH4 domain resulted in inhibition of ureteric bud branching. Therefore, the association of paxillin with bcl-2 BH4 domain may play a role during the early stages of nephrogenesis impacting ureteric bud branching.

MATERIALS AND METHODS

Transfection with Deletion Constructs—293 cells (human embryonic kidney cells) were transfected, using Lipofectin (Invitrogen), with murine bcl-2 deletion constructs in pcDNA3.1(−/−)MyelHis (tag in-frame with stop codon) (Invitrogen) containing 1–90 amino acids (aa), 1–140 aa, 1–190 aa, or vector (as a control). The inserts for the bcl-2 deletion constructs were prepared by PCR using the following primers: bcl-2 1–2 1–90 aa (ATTGAGCGCTTAGCAGGGCAAGCCGGGA and ATTCGGGATATCCCGGTTCAGGTACTCAG). The primers carry a NheI and EcoRV recognition sequence to facilitate subsequent cloning. The PCR products were examined on a 1% agarose gel to assess their integrity and expected size. For cloning, the PCR products were
BH4 peptides were synthesized at the University of Wisconsin Biotechnology Center with or without His$_e$ on the amino terminus. Some of these peptides were also synthesized with a carboxyl terminus TAT sequence (YGRKKRRQRRR-G). The resulting cell populations were immunoprecipitated with His$_e$ untagged or 10 μg His$_e$-tagged peptide was incubated with the Chariot reagent (Active Motif, Carlsbad, CA), and medium was added. All peptides were purified using a Spin Q column (Qiagen, Valencia, CA), digested with NheI and EcoRV, digested into the pCDNA3.1+–MyclHisA vector cut with the same enzymes, and transformed into Escherichia coli DH5α.

Bacterial colonies were screened by NheI and EcoRV digestion of mini-preps, and those with inserts of the appropriate size were sequenced using the Big Dye reagent (PerkinElmer Life Sciences) as described by the manufacturer. The samples were analyzed by the DNA sequencing facility at the University of Wisconsin Biotechnology Center (Madison, WI). The transfectants were grown in Dulbecco's modified Eagle's medium containing 5% fetal calf serum in the presence of 50 μg/ml streptomycin (Sigma), 50 units/ml nystatin (Sigma), and 500 μg/ml gentamicin (Invitrogen), and 50 units/ml G418.

Some experiments embryonic kidneys were incubated with vehicle or some experiments embryonic kidneys were incubated with His$_e$-tagged peptide. The nickel-nitrilotriacetic acid-agarose (200 μl; Qiagen) was washed with Hepes-buffered salt solution (20 mM Hepes, pH 7.4, 1–2 mg of the specific His$_e$-tagged peptide was bound to the agarose. The peptides described in Table I were synthesized at the University of Wisconsin Biotechnology Center with or without His$_e$ on the amino terminus. These peptides were used in the experiments described under “Results.”

As a source of protein NIH3T3 cells were chosen because they are mouse cells that express a significant amount of paxillin. NIH3T3 cell lysates were prepared in a modified radioimmune precipitation assay buffer containing 142.5 mM KCl, 5 mM MgCl$_2$, 10 mM Hepes, pH 7.4, 1% Nonidet P-40, and Complete protease inhibitor mixture (Roche Applied Science). The protein concentration was determined using a Bio-Rad DC protein assay. 500 μg of the specific His$_e$-tagged peptide of interest bound to Ni-agarose (Qiagen) at 4 °C and then washed three times with radioimmune precipitation assay buffer containing 10 mM imidazole. A portion of the sample was Western blotted using the following antibodies: anti-ILK (9E10; Sigma) and Western blotted for paxillin (BD Biosciences) essentially as described previously (10).

**Peptide Binding Assay**—The nickel-nitrilotriacetic acid-agarose (200 μl; Qiagen) was washed with Hepes-buffered salt solution (20 mM Hepes, 150 mM NaCl, pH 7.4), and 1–2 mg of the specific His$_e$-tagged peptide was bound to the agarose. The peptides described in Table I were synthesized at the University of Wisconsin Biotechnology Center with or without His$_e$ on the amino terminus. These peptides were used in the experiments described under “Results.”

| Peptides with or without a His$_e$ on the amino terminus |
|----------------------------------------------------------|
| bcl-2 BH4(7–31)                                      | TGYDNREIVMKYYIHYKLQSRQGYEWD |
| bcl-2 BH4(7–17)                                      | TGYDNREIVMKYYIHYKLQSRQGYEWD |
| bcl-2 BH4(17–31)                                     | KYYHKLQSRQGYEWD |
| bcl-2 BH2                                            | TW1QDNWDAFVELYG |
| bcl-xl                                               | DFLSYKLQSRQGYSN |
| bcl-w                                                | DFVPYKLQSRQGYVC |
| baf1f                                                | PPRFYHSLKPVSN |
| ced-9                                                | DYFTHRQNGMEN |
| S24D                                                 | KYYHKLDQSRQGYEWD |
| K22AR36A                                             | KYYHALQSRQGYEWD |
| Y21DY28D                                             | KYYHDKLRQSRQEDWN |
| Y21TF29SF                                           | KYYHKLRQSRQEDWN |

**TAT peptides**

| TAT peptides                                      |
|--------------------------------------------------|
| bcl-2 BH4$_{7–31}$–TAT                            |
| bcl-2 BH4$_{17–31}$–TAT                           |
| PECAM exon 14–TAT                                 |

**RESULTS**

Paxillin Interacts with an Amino-terminal Fragment of bcl-2—The molecular and cellular pathways by which bcl-2 mediates cell survival are not clearly understood. Apoptosis controls inappropriate cell positioning by requiring that differentiated epithelial cells remain in contact with their matrix for survival. Overexpression of bcl-2 protects cells from apoptosis caused by loss of adhesion. However, the mechanism by which bcl-2 protects cells as a result of loss of adhesion remains to be determined. I hypothesized that bcl-2 functions in part by interacting with paxillin. Support for this idea comes from my observation that bcl-2 and paxillin co-immunoprecipitate in embryonic kidney protein lysates. This interaction was shown to be direct by Far Western analysis (10).

To identify the domain in bcl-2 that interacts with paxillin, 293 cells were transfected with bcl-2 deletion constructs in pcDNA3.1(−)/MyclHisA (tag in-frame with stop codon). These constructs contained 1–90, 1–140, or 1–190 aa of bcl-2 (description of constructs is shown in Fig. 1A). Stable transfectants were obtained. The vector without an insert served as a control. The resulting cell populations were immunoprecipitated with anti-myc and Western blotted for paxillin. Fig. 1B demonstrates that paxillin co-immunoprecipitated with the first 90 aa of bcl-2 but not with the vector alone. I also observed association between paxillin and the other bcl-2 deletion constructs described in Fig. 1A (data not shown). Thus, expression of the first 90 amino acids of bcl-2 was sufficient for association with paxillin.
results. These experiments were repeated twice with similar results. Note that amino acids 1–90 from bcl-2 were sufficient for association with paxillin. These deletion constructs were transfected into 293 cells producing bcl-2 1–90, 1–140, or 1–190 aa. Cells were also transfected with vector alone as a control. In B, a population of transfectants were immunoprecipitated with anti-myc and Western blotted for paxillin. Note that amino acids 1–90 from bcl-2 were sufficient for association with paxillin. These experiments were repeated twice with similar results.

within the first 90 amino acids of bcl-2 is the BH4 domain (amino acids 10–30). To determine whether the BH4 domain was sufficient for association with paxillin, a His6-tagged bcl-2 BH4 domain peptide (amino acids 7–31) was synthesized. The His6-tagged bcl-2 BH4 peptide bound to nickel-agarose was incubated with NIH3T3 cell lysates, and the eluted peptides were Western blotted for paxillin. Fig. 2A demonstrates that the bcl-2 BH4 domain binds paxillin. 30 μg of NIH3T3 protein lysate and a comparable amount from the original starting material bound to BH4- or BH2-agarose was Western blotted for paxillin. Approximately 75% of paxillin in NIH3T3 cell lysates binds to the BH4 domain peptide (compare 3T3 lysate to BH4 lanes) under these experimental conditions (Fig. 2A). Furthermore, paxillin did not associate with a His6-tagged bcl-2 BH2 domain peptide (Fig. 2A), demonstrating that association between paxillin and bcl-2 was specific for the BH4 domain. Addition of excess untagged BH4 peptide decreased the amount of paxillin associating with the His6-tagged bcl-2 BH4 peptide (Fig. 2B).

Paxillin Interacts with the BH4 Domain from Other bcl-2 Family Members—To narrow the portion of the bcl-2 BH4 domain that was essential for paxillin binding, I next synthesized His6-tagged peptides to bcl-2 amino acids 7–17 and 17–31. Fig. 3 demonstrates that paxillin associated with amino acids 17–31 but not amino acids 7–17 from the bcl-2 BH4 domain (Fig. 3, A and B). The next question asked was what amino acids were critical for binding. Instead of mutating each amino acid individually to determine which one(s) were important, the diversity of the BH4 domains within the bcl-2 family members was exploited. Mammalian as well as non-mammalian (ced9 and balf1) bcl-2 family members contain BH4 domains. Although some similarity exists between these domains, they are not identical (Fig. 3C). His6-tagged peptides to the matching amino acids 17–31 in the bcl-2 BH4 domain from bcl-xL, bcl-w, balf1, and ced9 were synthesized. The sequences for the domain with the actual peptides synthesized and association with paxillin are described in Fig. 3. Paxillin associated with the BH4 domains of more closely related bcl-2 family members, bcl-xL and bcl-w. Some binding of paxillin to the balf1 BH4 domain was observed, but little if any association was observed with another non-mammalian homologue, ced9 (Fig. 3).

Substitutions in the bcl-2 BH4 Domain Affect Association with Paxillin—Paxillin associated with closely related mammalian bcl-2 family members and to a lesser degree with non-mammalian homologues (Fig. 3). The amino acid sequence differences between the BH4 domains of the bcl-2 family members were examined and compared with their ability to associate with paxillin. Next, peptides were synthesized with substitutions that could potentially affect binding to paxillin. The initial substitutions made were S24D, K22A/R26A, and Y21D/Y28F. As shown in Fig. 4A, changing amino acids 90 and 97 to tyrosines blocked paxillin binding. Even when a more conservative substitution, Tyr→Phe, association with paxillin did not occur (Fig. 4B). The inability of paxillin to bind to the BH4 domain when tyrosines 21 and 28 are substituted with phenylalanine or aspartic acid could explain why no significant association between paxillin and the ced9 BH4 domain, which lacks these tyrosines, was observed. To address this possibility the ced9 BH4 domain was synthesized with histidine 90 and methionine 97 replaced with tyrosines. As shown in Fig. 4B, changing amino acids 90 and 97 to tyrosines was sufficient to facilitate paxillin association with the ced9 BH4 domain. Thus, these data indicate that the unphosphorylated tyrosines spaced 7 amino acids apart in the bcl-2 BH4 domain may play a role in the association with paxillin. These data are summarized in Fig. 4C. Even the seemingly minor substitution Y21F/Y28F may cause significant topographical changes in the BH4 structure such that association with paxillin is inhibited.

**Fig. 2.** Paxillin associates with the bcl-2 BH4 domain. In A, a His6-tagged bcl-2 BH4 or BH2 domain peptide bound to nickel-agarose was incubated with NIH3T3 cell lysate. 30 μg of NIH3T3 protein lysate and a comparable amount from the original starting material bound to BH4 or BH2 were Western blotted for paxillin. Under these conditions, ~75% of paxillin in NIH3T3 cell lysates bind to the BH4 domain peptide (compare 3T3 lysate to BH4 lanes) as determined by densitometry. In B, excess untagged BH4 peptide was added. These experiments were repeated three times with similar results.
Paxillin Interacting with bcl-2 Does Not Affect Its Association with Other Proteins—To examine whether other proteins that normally bind paxillin can associate when paxillin binds the bcl-2 BH4 domain, the His6-tagged BH4 peptide bound to nickel-agarose was incubated with NIH3T3 cell lysate. The eluted protein(s) were Western blotted for paxillin. B demonstrates similar experiments utilizing the BH4 domain from bcl-2 family members. C indicates the peptides used and summarizes their general ability to associate with paxillin. These experiments were repeated three times with similar results.

Embryonic kidneys from embryonic day 11.5 (E11.5) pregnant normal mice were grown in the presence of vehicle or 20 μM BH4 bcl-2-TAT peptide (DNREIVMKYHYKLQRYEYNKAYGRKRRQRGRG) for 4 days at 37 °C. To ensure penetration of the peptide I linked a highly basic peptide derived from the human immunodeficiency virus TAT protein (YGRKKRRQRRRG) to the carboxyl terminus. This sequence is sufficient to enable translocation of peptides across cell membranes (13, 14).

Incubation with the BH4 Domain Peptide Inhibits Ureteric Bud Branching—To begin to examine the role the bcl-2 BH4 domain plays in vivo, embryonic kidney (metanephric) organ culture was utilized. Embryonic kidneys grown in organ culture underwent the complex early events in renal differentiation in a manner similar to that observed in the embryo. My laboratory previously used this model system to examine abnormal kidney development in bcl-2−/− and Osf+ mice (11, 12). To minimize the variability between kidneys originating from different embryos, comparisons were made between kidneys from the same embryo (right and left kidneys).

Fig. 6A demonstrates a photomicrograph and corresponding H&E sections of embryonic kidneys incubated in the presence or absence of 20 μM BH4 bcl-2-TAT peptide. After 4 days in organ culture, growth was noticeably impaired in embryonic kidneys incubated with the BH4 bcl-2-TAT peptide. Next, H&E sections from these embryonic kidneys were examined. Components of the ureteric bud and surrounding metanephric blas-
the His6-tagged bcl-2 BH4 domain peptide. This lipid-based culture. The Chariot protein delivery agent was used to deliver with the BH4 peptide in embryonic kidneys incubated in organ embryonic kidney organ culture require tyrosines 21 and 28 in to paxillin as well as the inhibitory effect of the BH4 peptide in these data suggest that both binding of the bcl-2 BH4 domain Y21D/Y28D-mutated peptide-incubated embryonic kidneys. To visualize the ureteric bud, embryonic kidneys were whole mount-stained with D. bifilorus agglutinin (Fig. 6B). Embryonic kidneys incubated with the bcl-2 BH4 domain exhibited substantially less ureteric bud branching. The embryonic kidneys also appear slightly smaller than control kidneys. I repeated these experiments with bcl-2 BH4-TAT-TAT peptide with similar results (data not shown). This was not merely due to the presence of the TAT peptide because incubation of embryonic kidneys with a PECAM-1 exon 14-TAT peptide did not affect ureteric bud branching or kidney morphogenesis. To further ensure that the observed effect was attributable to the bcl-2 BH4 domain, I repeated these experiments using the Chariot reagent to deliver untagged bcl-2 BH4 peptide to the embryonic kidneys. Similar results were observed as those shown with the BH4 TAT-labeled peptides (data not shown). As an additional control, the BH4 Y21D/Y28D-mutated peptide was used. The BH4 Y21D/Y28D peptide did not associate with paxillin in the binding experiments performed above (Fig. 4). Morphogenesis and ureteric bud branching were similar in control and BH4 Y21D/Y28D-mutated peptide-incubated embryonic kidneys. These data suggest that both binding of the bcl-2 BH4 domain to paxillin as well as the inhibitory effect of the BH4 peptide in embryonic kidney organ culture require tyrosines 21 and 28 in bcl-2 BH4 domain. The next question asked was whether paxillin associated with the BH4 peptide in embryonic kidneys incubated in organ culture. The Chariot protein delivery agent was used to deliver the His6-tagged bcl-2 BH4 domain peptide. This lipid-based protein delivery system efficiently translocates macromolecules into cells. E11.5 embryonic kidneys were grown on filters with similar results. To determine whether cell viability declined in the presence of the BH4 peptide pyknotic nuclei were scored. A 3-fold increase in pyknotic cells (3% in controls versus 9% in BH4-incubated) was observed in embryonic kidneys incubated with the BH4 peptide.

To summarize, the results presented here indicate that the BH4 domain of bcl-2 protects cells from apoptosis by interacting with other proteins. Gaining a better understanding of the molecular and cellular pathways by which bcl-2 mediates cell survival is essential for determining its role during development. bcl-2 co-immunoprecipitates with paxillin and FAK in lysates from embryonic kidneys. Utilizing Far Western analysis, the interaction between bcl-2 and paxillin was shown to be direct, whereas its interaction with FAK was indirect. Here, the association between bcl-2 and paxillin has been investigated further. The major findings are as follows: 1) The bcl-2 BH4 domain, specifically amino acids 17–31, interact with paxillin. 2) Mutating tyrosines 21 and 28 in the BH4 domain of bcl-2 abolishes binding to paxillin. Tyrosines at these locations are conserved in the more closely related bcl-2 family members bcl-w and bcl-x. 3) Replacing histidine 90 and methionine 97 in the ced9 BH4 domain with tyrosines facilitated association with paxillin. 4) Incubation of embryonic kidneys in organ culture with a BH4 domain peptide inhibits ureteric bud branching and morphogenesis. In contrast, incubation of embryonic kidneys with a BH4 Y21D/Y28D peptide does not significantly affect ureteric bud branching. Therefore, bcl-2 interaction with paxillin through its BH4 domain may be essential during nephrogenesis.

Paxillin is a 68-kDa focal adhesion protein that interacts with focal adhesion and signal transduction proteins. The paxillin amino terminus contains binding sites (leucine-rich motif; LD motifs) for FAK, Csk, Src, PYK2, and vinculin (15–19), while the carboxyl terminus has four LIM domains targeting it to focal adhesions. Paxillin is required during embryonic development and acts as a cytoplasmic effector for some fibronectin receptors (20). Moreover, fibronectin expression is important for cleft formation during branching morphogenesis in the salivary gland (21). Paxillin-deficient mice are abnormal by E8 (prior to kidney development) (20, 22). Paxillin can function during the regulation of migration, cell spreading, and phosphorylation. This may be the direct or indirect result of interaction with proteins such as FAK, Crk, Cas, PTP-PEST, and Csk. ILK, PTP-PEST, Crk, Cas, FAK, and vinculin associated with bcl-2 BH4 peptide-bound paxillin (Fig. 5). Thus, targeting paxillin to specific regions of a particular organ during development could have profound implications as to the microcosm of signaling molecules available in that area.

bcl-2 is widely expressed during development and becomes restricted upon maturation in many tissues. The kidney demonstrates this quite well. During nephrogenesis apoptosis tends to be inversely correlated with bcl-2 expression (23). bcl-2 is highly expressed early in the embryonic kidney, and its level decreases significantly at later times such that its expression is normally low in the postnatal kidney (24). In the developing kidney bcl-2 is expressed in the ureteric bud (24) and in epithelial condensates of the metanephric blastema (23). However, bcl-2 is not expressed in uninduced mesenchyme (23). Loss of bcl-2 dramatically affects kidney development. Mice deficient in bcl-2 (bcl-2−/−) develop renal hypoplasia/cystic dysplasia. Kidneys in these mice undergo fulminant apoptosis of the metanephric blastema during early embryogenesis (E12), and the renal epithelial cells do not complete terminal differentiation in the postnatal kidney (10, 12, 25–28). Perhaps early embryonic expression of bcl-2 and its association with paxillin facilitates morphogenesis by supporting survival of precursor cells allowing them to be less adherent and more migra-

Fig. 5. Paxillin-associated proteins remain in the presence of the BH4 peptide. The His6-tagged BH4 peptide bound to nickel-agarose was incubated with NIH3T3 cell lysates. The eluted proteins were Western blotted for ILK, PTP-PEST, Crk, Csk, FAK, and vinculin. These experiments were repeated twice with similar results.
Proper regulation of apoptosis is essential for nephrogenesis to proceed normally. Although bcl-2 is known to be highly expressed in the murine kidney following induction, its normal function is not completely understood. Loss of bcl-2 impairs ureteric bud branching and causes fulminant apoptosis of the metanephric blastema (12). Whether bcl-2 merely acts as a survival factor or plays a more active role by associating with

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*Fig. 6. The BH4 domain disrupts nephrogenesis.* Embryonic kidneys from E11.5 mice were grown in the presence of vehicle or 20 μM BH4 bcl-2-TAT peptide for 4 days. Comparisons were made between kidneys from the same embryo (right and left kidneys). A shows a photomicrograph and corresponding H&E sections of embryonic kidneys. To visualize the ureteric bud, embryonic kidneys were whole mount-stained with *D. biflorus* agglutinin in B. Micrographs are representative of greater than 8 sets of kidneys. These experiments were repeated twice with similar results.

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*Figures are cited as follows:*

1. C. M. Sorenson, unpublished data.
paxillin as part of a differentiation cascade during nephrogenesis remains to be seen. Enhanced cell survival has two consequences. The obvious one is that it maintains the progenitor cell population. The less obvious one is that it allows progenitor cells to disengage from the substratum and to migrate, resulting in the formation of nephron segments. The notion that bcl-2 can influence cell migration is supported by the fact that bcl-2 expression is regulated by interaction of integrins with components of the basement membrane (9, 29). Thus, the early embryonic expression of bcl-2 and its association with paxillin could facilitate morphogenesis by supporting survival of precursor cells allowing them to be less adherent and therefore more able to migrate without the threat of apoptosis. This is consistent with the fulminant apoptosis observed in embryonic kidneys incubated with the BH4 peptide. The BH4 domain-incubated embryonic kidneys appear similar to embryonic kidneys incubated with the BH4 peptide. The BH4 domain-incubated embryonic kidneys appear similar to embryonic kidneys incubated with bcl-2 BH4 Y21D/Y28D peptide to affect ureteric bud branching. It is tempting to speculate that the association of bcl-2 with paxillin supports branching morphogenesis by circumventing the need for integrin signaling for survival. An alternative explanation is that the bcl-2 interaction with paxillin recruits other important players in integrin signal transduction that associate with paxillin, positively influencing differentiation. Therefore, disruption of ureteric bud branching may be most sensitive to the BH4 domain early when bcl-2 expression is high and before the signaling machinery orchestrating branching is firmly in place.

The role bcl-2 plays as a survival factor appears to be rather complex, perhaps utilizing different pathways for specific purposes. For example, the manner in which bcl-2 facilitates survival of mesenchyme and ureteric bud cells during nephrogenesis may not be identical. The mode of morphogenesis and the organ itself may predetermine the method by which bcl-2 or other family members act as survival factors. Therefore, gaining a better understanding of the interrelationship between cell survival and adhesive processes will further our insight into nephrogenesis.

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Christine M. Sorenson

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