The Development-associated Cleavage of Lens Connexin 45.6 by Caspase-3-like Protease Is Regulated by Casein Kinase II-mediated Phosphorylation*

Xinye Yin, Sumin Gu, and Jean X. Jiang‡

From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229-3900

Gap junctions are important in maintaining lens transparency and metabolic homeostasis. In this paper, we report that the gap junction-forming protein, connexin (Cx) 45.6, was specifically truncated during lens development and that the majority of the truncated fragments were located in the differentiated lens fibers. When isolated lens membranes were treated by caspase-3, the truncated fragments of Cx45.6 were reproduced, and this truncation occurred at the COOH terminus of Cx45.6. Moreover, when primary lens cells were treated with apoptosis-inducing reagents, Cx45.6 was cleaved similarly as the in vitro treatment by caspase-3, and this cleavage was blocked by a caspase-3 inhibitor. These results suggest that caspase-3 is responsible for the development-associated cleavage of Cx45.6.

The cleavage site of Cx45.6 was identified between amino acid residues Glu367 and Gly368. We have shown previously that Ser363 is an in vivo phosphorylated site by casein kinase II, and this phosphorylation leads to a rapid turnover of Cx45.6. Interestingly, we found here that when Ser363 was phosphorylated by casein kinase II, the cleavage of Cx45.6 catalyzed by caspase-3 was inhibited. This study, for the first time, demonstrates that a connexin can be a direct target of an apoptotic protease and that cleavage by caspase-3-like protease leads to the development-associated truncation of a lens connexin. Finally, caspase-3-mediated cleavage can be regulated by casein kinase II-mediated phosphorylation, suggesting that Cx45.6 turnover and specific cleavage by caspase-3-like protease is alternatively modulated.

The vertebrate lens is one of the most important model systems used in the study of the function and regulation of gap junctions. The lens is an avascular organ composed of an anterior epithelial cell layer and highly differentiated fibers ranging from the outer cortex toward the central core region. The terminal differentiation and aging of lens fibers are marked by dramatic morphological changes. As new cells arise on the outside of the lens, older cells are pushed inward where nuclei and organelles are lost (1). Because these cells are never lost from the lens, the most central cells are as old as the organism itself. The survival of lens cells relies on the intercellular communications between these cells and the cells at the lens surface through a large network of gap junctions that facilitate the exchange of ions and metabolites throughout the organ (2, 3).

Gap junctions are intercellular channels between two adjacent cells, which allow passage of small molecules (≤1000 Da) such as small metabolites, ions, and second messengers. The structural components of gap junctions are the members of a protein family called connexins, which consist of four conserved transmembrane domains and two extracellular loops, whereas their cytoplasmic regions are unique. The COOH terminus, the most variable region among connexins, contains several kinase and protease consensus sequences. Connexins are highly dynamic proteins that undergo rapid turnover both in cell lines (4) and in animal organs (5–7). The degradation has been identified to undergo either through the lysosome or proteasome pathway (8–12). Phosphorylation has been demonstrated to play an important role in regulation of gap junction stability and turnover (13–16). We have recently shown that an in vivo phosphorylated lens connexin undergoes a faster turnover than its unphosphorylated counterpart (17).

Differentiating lens fibers share a number of morphological and biochemical characteristics with cells undergoing apoptosis such as nucleus degeneration, loss of organelles, and activation of members of a cysteine protease family named caspase (18–21). However, unlike apoptotic cells, which are rapidly digested, the organelle-free lens fibers retain their basic cell integrity and metabolism throughout the lifetime of the organ. Poly(ADP-ribose) polymerase, an enzyme involved in DNA repair and maintenance of genomic integrity, is cleaved by caspases in the developing lens (18). Caspase-3 is predominantly activated during staurosporine-induced apoptosis in lens cells (22). The function of caspases has been reported to be responsible for nucleus degradation during the later stage of lens fiber differentiation (18). Although activation of caspases has been demonstrated during lens fiber differentiation, only a couple of caspase substrates have been identified, such as cytoskeletal proteins named α- and β-spectrins (21).

Connexin (Cx) 45.6 is one of the connexins expressed in chick lens fibers (23, 24). Cx45.6, which is different from the other two chick lens connexins, Cx43 and Cx50, seems to be involved in lens development and differentiation. Mutations in Cx50, the mammalian ortholog of Cx45.6, have been identified in human cataract families (25, 26). Mice deficient in Cx50 not only develop cataracts, but also exhibit microphthalmia with a decrease of lens size (27). Primary cultures of chick lens closely

* This work was supported by National Institutes of Health Grant EY-12085 (to J. X. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900. Tel.: 210-567-3796; Fax: 210-567-6595; E-mail: jiangj@uthscsa.edu.

1 The abbreviations used are: Cx, connexin; CKII, casein kinase II; CEF, chicken embryonic fibroblast; DMEM, Dulbecco’s modified Eagle’s medium; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; Z, benzylxoyycarbonyl; FMK, fluoromethyl ketone; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid; E, embryonic day; P, postnatal day.

This paper is available on line at http://www.jbc.org
mimic in vivo lens cell differentiation processes in which the monolayer of the lens epithelial cells differentiate into structures called “lentoids” displaying features of differentiated lens fibers (28, 29). We have recently shown that overexpression of Cx45.6 in lens primary cultures stimulates lens cell differentiation and formation of fibers (30). Additionally, Cx45.6 is subjected to post-translational modification in vivo. Cx45.6 is a phosphoprotein (23, 31). We have recently shown that Cx45.6 is phosphorylated in vivo by CKII at Ser361 located at the COOH-terminal region, and this phosphorylation facilitated the turnover of Cx45.6 (17). A previous report shows that Cx50, the ovine ortholog of Cx45.6, is also truncated at the COOH terminus in the center of the lens by a protease called calpain (32). Although post-translational modifications, such as phosphorylation and specific truncation of lens connexins, have been investigated previously, the physiological significance and regulatory mechanism of these modifications in lens differentiation and development are less certain. In this report, we identified a specific proteolytic cleavage of Cx45.6 associated with lens development. Our experimental evidence suggests that the protease involved in this cleavage is caspase-3, an apoptotic protease. Moreover, the cleavage site by caspase-3 in Cx45.6 was identified to be close to the phosphorylated site by CKII. This specific phosphorylation protects Cx45.6 from cleavage by caspase-3. This is the first report showing that the apoptotic protease, caspase-3, is responsible for cleavage of a connexin, and this specific cleavage is regulated by a specific phosphorylation.

**EXPERIMENTAL PROCEDURES**

**Materials—**Fertilized chicken eggs were obtained from SPAFAS (Rockland, IL) and Tyson Hatchery (Gonzalez, TX). Cx45.6 and caspase-2 were obtained from Sigma. Caspase-3 inhibitor Z-DEVD-FMK was from Calbiochem. [γ-32P]ATP and [35S]methionine were from PerkinElmer Life Sciences. Tissue culture reagents were purchased from Life Technologies, Inc. SDS-PAGE standards were from Bio-Rad. QuikChange™ Site-directed Mutagenesis kit was obtained from Stratagene (La Jolla, CA). Chemiluminescence kit, ECL, was from Amersham Pharmacia Biotech. X-Omat AR film was obtained from Eastman Kodak.

**Preparation of GST Fusion Proteins and Immunoaffinity Purification of Anti-Cx45.6 Antibody—**Bacterial fusion proteins containing GST fused with various portions of Cx45.6 were prepared as described (23, 33). The fusion proteins generated included the following: GST fused with partial COOH terminus of Cx45.6, GST-Cx45.6F1 (amino acids 307–389) and its corresponding site mutants, GST-Cx45.6F1(D364A) and GST-Cx45.6F1(E367A); GST fused with entire Cx45.6 amino acids 98–400; GST-Cx45.6F2 (amino acids 237–400); and GST-Cx45.6F2 (amino acids 98–148). Briefly, DNA fragments containing various portions of Cx45.6 or its mutants were produced by PCR with specific oligonucleotide primers. The codon GAT encoding Asp364 was changed to GCA encoding Ala in one of the primer sequences required for mutant synthesis. Each DNA fragment generated was inserted into the expression vector pGEX-2T. The recombinant fusion proteins were expressed in Escherichia coli, induced by isopropyl-thio-β-D-galactoside, and isolated with GST beads. To ensure the correct sequence all constructs generated were sequenced at the Institutional DNA Sequencing Facility. Fusion protein containing GST plus intracellular loop portion of Cx45.6 was used to raise polyclonal antisera in rabbits (Porono Rabbit Farm and Laboratory Inc, Canadensis, PA). The antisera generated were immunoaffinity-purified by passage through two Sepharose CL-4B columns, GST-conjugated and GST-Cx45.6 fusion protein-conjugated, respectively, as described (23, 33).

**SDS-PAGE and Western Blotting—**The fusion proteins and crude membrane samples were loaded in each lane of a 10% SDS-PAGE. For Western blotting, 10 µg total of SDS-PAGE protein were transferred to nitrocellulose membranes according to the method of White et al. Membranes were probed with the affinity-purified preimmune or Cx45.6 antibody (1:500 dilution). The primary antibodies were detected either with alkaline phosphatase-conjugated secondary anti-rabbit antisera or peroxidase-conjugated secondary anti-rabbit antisera and followed by chemiluminescence reagent kit (ECL) (Amersham Pharmacia Bio-
tech) according to the manufacturer’s instruction. The membranes were exposed to X-Omat AR films (Eastman Kodak) and detected by fluorography.

**Preparation of Retroviral Constructs and High Titer Retroviruses Carrying Wild Type and Mutant Cx45.6—**Recombinant retroviruses were produced from RCAS(A)-Cx45.6(E367A) and RCAS(A)-Cx45.6(E367D), which have been described previously (35, 36). Briefly, DNA fragment containing the wild type Cx45.6 was made by PCR and was constructed into the retroviral vector RCAS(A) as described (17, 36). With the wild type RCAS(A)-Cx45.6 DNA construct as a template, retroviral constructs of Cx45.6 mutants containing point mutations were generated with the QuikChange™ Site-directed Mutagenesis kit according to the manufacturer’s instructions. The codon alteration for site mutants, RCAS(A)-Cx45.6(D364A) and RCAS(A)-Cx45.6(E367D), were described above. RCAS(A)-Cx45.6(E367D) was made by changing codon GAA encoding Glu367 to GAT encoding Asp. All constructs were generated were sequenced at the Institutional DNA Sequencing Facility. The high titer retroviruses (10⁻⁵ to 10⁻⁶ colony forming units/ml) containing the wild type and the mutant RCAS(A)-Cx45.6 constructs were prepared as we have described previously (35, 36).

**Isolation of Cell Membranes from Lens or CEF Cells and in Vitro Cleavage by Caspase-3—**The crude cell membranes were isolated and prepared as described (37). Briefly, lenses from embryonic day 9 or CEF cells infected with retroviruses were lysed in lysis buffer (5 mM Tris, pH 8.0, 0.5 mM EDTA, 0.5 mM sucrose, and 5 mM glycerol) with and without 0.3 µg of caspase-3 at various times. Reactions were terminated by the addition of electrophoresis sample buffer (50 mM Tris, pH 6.8, 1% SDS, 2% b-mercaptoethanol, and 35% glycerol) and boiled for 5 min.

**Preparation of Primary Lens Cultures, Treatment with Apoptosis-inducing Reagents, and Inhibition of Caspase-3 Activity—**Primary lens cultures were prepared according to the procedure described previously (17, 38). Briefly, chick lenses dissected from embryonic day 11 embryos were ruptured, and the cells that dissociated from the lenses were plated at 1 x 10⁵ cells per 35-mm tissue culture plates in medium 199 plus 10% fetal bovine serum. Eight days after cell plating, primary cultures were treated either with 5 µM staurosporine dissolved in culture medium or with 2 mM EGTA for 4.5 h at 37 °C. When caspase-3 inhibitor Z-DEVD-FMK was added, caspase-3 activity was inhibited with this inhibitor for 1 h before exposure to staurosporine or EGTA.

**Culture of CEF Cells, Retroviral Expression, and Treatment with Apoptosis-inducing Reagents—**CEF cells were plated at 1 x 10⁵ cells in 35-mm tissue culture plates with DMEM plus 10% fetal calf serum and 5% CO₂. The 2nd day after cell plating CEF cells were infected with high titer recombinants (5 µl/dish) carrying either the wild type or the mutant Cx45.6 cDNAs. When CEF cells reached confluence, the cultures were collected either for membrane preparation or for treatment with apoptosis-inducing reagents. CEF cells were treated in the presence of 1 mM staurosporine for 8 h at 37 °C.

**Phosphorylation of Cx45.6 by CKII, Radioactive Labeling of Lens Organ, in Vitro Cleavage by Caspase-3, and Immunoprecipitation—**The isolated embryonic lens membranes, cultures were preincubated with this inhibitor for 1 h before exposure to staurosporine or EGTA.

**Cleavage of Lens Cx45.6 by Caspase-3-like Protease**
RESULTS

Development-associated Cleavage of Lens Cx45.6—Chick lenses at embryonic (E) days 8, 12, 15, and 18 and postnatal (P) days 1, 15, 30, and 60 were immunoblotted with affinity-purified Cx45.6 antibody that recognized the intracellular loop region of Cx45.6. The full-length Cx45.6 was phosphorylated in vivo and migrated as three close bands at the positions of 53, 56, and 58 kDa (Fig. 1) as demonstrated previously (23). At E8, a full-length Cx45.6 was solely present in the lens (Fig. 1, lane 1). Starting at E12, an additional band appeared migrating at around 46 kDa (empty arrowhead) (Fig. 1, lane 2) and its concentration level increased during the development of the lens as shown at E15 (Fig. 1, lane 3), E18 (Fig. 1, lane 4), and postnatal stages (Fig. 1, lanes 5–8). In addition, a 48-kDa fragment (solid arrowhead) closely migrating with the 46-kDa fragment appeared at E15, which is a lesser amount than 46-kDa fragment at embryonic stages. The level of this fragment, however, increased dramatically at the later developmental stages and reached a similar level as the 46-kDa fragment as when the cleavage of Cx45.6 was observed. To determine whether caspase-3 is the protease responsible for the cleavage, embryonic lens membranes were incubated with caspase-3. As shown in Fig. 3B, after 2-h incubation, the full-length Cx45.6 was partially cleaved into fragments around 46 kDa (Fig. 3B, lane 1, arrowhead), which migrated to the same position as the truncated Cx45.6 of the lens membrane from the newborn chick (Fig. 3B, lane 5, arrowhead). After 16-h incubation with caspase-3, the majority of the full-length Cx45.6 proteins was cleaved (Fig. 3B, lane 3, arrowhead).

These results indicate that Cx45.6 may be a direct substrate for caspase-3. Alternatively, Cx45.6 can be cleaved by other protease(s) present in the lens membrane that could be activated by caspase-3. To examine these possibilities further, purified fusion proteins containing GST fused with portions of Cx45.6 were used for in vitro caspase-3 enzymatic digestion. The fusion partners of Cx45.6 include the following: the intracellular loop of Cx45.6 (GST-45.6L) (amino acids 98–148) (Fig. 3C, lanes 3 and 4), partial COOH terminus (GST-45.6P2) (amino acids 237–400) (Fig. 3C, lanes 7 and 8). These fusion proteins, together with the GST protein itself (Fig. 3C, lanes 1 and 2), were treated with caspase-3. Both GST-45.6 F1 (Fig. 3C, lane 6) and GST-45.6 F2 (Fig. 3C, lane 8) were cleaved by caspase-3 (arrowheads), whereas GST protein (Fig. 3C, lane 2) and GST-45.6 F1 (Fig. 3C, lane 4) were not cleaved. These results suggest that Cx45.6 is a substrate of caspase-3 and that the cleavage site is located within the COOH terminus between amino acids 307 and 389.
Development-associated Truncation of Cx45.6 Was Reproduced by a Caspase-3-like Protease Following the Treatment of Apoptosis-inducing Reagents in Lens Cells—To examine whether Cx45.6 can be cleaved by caspase-3 in lens cells, primary lens cultures were incubated with apoptosis-inducing reagents staurosporine (Fig. 4, lane 2) (22) or EGTA (Fig. 4, lane 4) (40). These two compounds are known chemical inducers for caspase-mediated apoptosis (22, 40). Treatments by both reagents regenerated fragments around 46 kDa (Fig. 4, lanes 2 and 4, arrowhead). The cleavage was significantly inhibited by Z-DEVD-FMK, a caspase-3 inhibitor (Fig. 4, lane 5). The results confirm that caspase-3 is likely to be involved in the cleavage of Cx45.6 in vivo.

Caspase-3 Cleaved Amino Acid Residues Glu367 and Gly368—The amino acid sequence Asp364-Glu-Val-Glu367-Gly was identified in the COOH-terminal region of Cx45.6 (see Fig. 5A). This sequence is similar to a known caspase-3 consensus sequence (41) with the exception of substituting Asp with Glu at position 367. If cleavage by caspase-3 occurs between Glu367 and Gly368, the predicted molecular weight of one of the cleaved fragments would match that of the 46-kDa fragment. The two aspartate residues in the consensus sequence of caspase-3 are essential, and cleavage cannot occur if either residue is altered to alanine (42). Since glutamate like aspartate is an acidic residue, this segment of the sequence in Cx45.6 is a potential target for caspase-3. As illustrated in Fig. 5A, mutants were constructed with alterations in the conserved amino acid residues corresponding to the consensus sequence of caspase-3. Fusion proteins GST-Cx45.6F1, and its mutants GST-Cx45.6F1(D364A) and GST-Cx45.6F1(E367A) were subjected to caspase-3 cleavage in vitro (Fig. 5B). GST-Cx45.6F1 was cleaved by caspase-3 into two closely migrating bands (Fig. 5B, lane 2). Both GST-Cx45.6F1(D364A) (Fig. 5B, lane 4) and GST-Cx45.6F1(E367A) (Fig. 5B, lane 6) exhibited resistance to cleavage by caspase-3. These data suggest that either Asp364 or Glu367 seems to be crucial in the cleavage by caspase-3.

The COOH terminus of Cx45.6 in GST fusion protein may not have similar conformation as compared with the one in the membrane-spanning full-length molecule. Therefore, full-length Cx45.6 and its mutants carrying alterations of conserved residues corresponding to caspase-3 (see Fig. 5A) were expressed in CEF cells through retroviral infection, and the isolated cell membranes were subjected to caspase-3 treatment (Fig. 5C). Similar to the results shown for fusion proteins, mutations in D364A and E367A prevented cleavage of Cx45.6 by caspase-3 (Fig. 5C, lanes 4 and 6). Furthermore, the mutation of E367D, producing a cleavage sequence in Cx45.6 identical to the consensus sequence for caspase-3, increased cleavage (Fig. 5C, lane 8). The ratio of the fragments around 46 kDa to the full-length form of Cx45.6(E367D) (Fig. 5C, lane 8) is greater than that to the full-length wild type Cx45.6 (Fig. 5C, lane 2).

Similar results were observed when CEF cells expressing wild type and mutant Cx45.6 were treated by the apoptosis-inducing reagent staurosporine (Fig. 5D). Neither Cx45.6(D364A) nor Cx45.6(E367A) was cleaved (Fig. 5D, lanes 4 and 6), whereas the wild type Cx45.6 was (Fig. 5D, lane 2). In comparison to the wild type Cx45.6, the cleavage of Cx45.6(E367D) was further enhanced (Fig. 5D, lane 8). Together, the results obtained from fusion proteins and full-length Cx45.6 expressed in cells suggest that the cleavage site of Cx45.6 by caspase-3 is between Glu367 and Gly368.

Inhibition of Caspase-3-catalyzed Cleavage of Cx45.6 by Phosphorylation of Cx45.6 by CKII—We have reported that Ser363, an amino acid residue close to the caspase-3 cleavage site, is phosphorylated in vivo by CKII (17). Phosphorylation of certain substrates has been shown to be important in regulating their cleavage by caspase-3 (42, 43). To determine whether phosphorylation at Ser363 affects the ability of Cx45.6 to serve as a substrate for caspase-3, Cx45.6 in the membranes of embryonic lenses was treated in the presence of CKII and followed by proteolytic digestion by caspase-3 (Fig. 6, lane 2). The results indicate that Cx45.6 phosphorylated by CKII was protected from caspase-3 digestion. To ensure that Cx45.6 in both full-length and truncated forms could be immunoprecipitated, embryonic lenses were metabolically labeled with [35S]methionine and chased in non-radioactive medium immediately (Fig. 6, lanes 3 and 4) and after 6 h of incubation (Fig. 6, lanes 5 and 6). The isolated membranes labeled with [35S]methionine were
in CEF cells were treated with (lanes 2, 4, 6, and 8), were treated with (lanes 2, 4, and 6) and without (lanes 1, 3, and 5) caspase-3. The cleaved fragments are indicated by an arrowhead. C, exogenous full-length Cx45.6 (lanes 1 and 2) and its corresponding site mutants, Cx45.6(D364A) (lanes 3 and 4), Cx45.6(E367A) (lanes 5 and 6), and Cx45.6(E367D) (lanes 7 and 8), were expressed in CEF cells through retroviral RCAS(A) infection. Crude membranes of Cx45.6 expressed in CEF cells were treated with (lanes 2, 4, 6, and 8) and without (lanes 1, 3, 5, and 7) caspase-3, and the fragments resulting from digestion reaction are indicated by an arrowhead. D, CEF cells expressing wild type and mutant Cx45.6 through retroviral RCAS(A) infection were treated with (lanes 2, 4, 6, and 8) and without (lanes 1, 3, 5, and 7) caspase-3. The cleaved fragments are indicated (arrowhead).

Our previous studies (17) have shown that Cx45.6 is phosphorylated by CKII at Ser363, it becomes unstable and possibly undergoes degradation mediated through the proteasome pathway (17). The CKII phosphorylation site Ser363 is located within a PEST domain (17, 44), and this domain has been reported to be associated with a rapid protein turnover via proteasome-mediated degradation (45). Additionally, phosphorylation is also known to signal protein degradation by proteasome (46). When Ser363 is not phosphorylated by CKII, Cx45.6 renders itself into a substrate for caspase-3, which cleaves at Glu367 at the COOH terminus of Cx45.6 and generates truncated fragments of Cx45.6. These fragments were localized to the central core region of the lens.

We observed two fragments derived from Cx45.6 in the size of 46 and 48 kDa. These fragments were accumulated during lens development. The 46-kDa fragment was predominant during the embryonic stage, whereas the level of the 48-kDa fragment increased and reached a similar level as compared with the 46-kDa form at the postnatal stage. Formation of two fragments was also observed when GST-Cx45.6.6F1 was treated with caspase-3. However, mutation of a potential cleavage site in GST-Cx45.6F1 blocked the production of either fragment, suggesting that this site initiates generation of these two fragments. Because of the closeness of their migration, these two fragments sometimes were less distinctive on the SDS-PAGE. We also observed a potential third band larger than 48 kDa at P60 samples, and this band could be generated from additional cleavage or certain type of posttranslational modifications.

Based on our experimental evidence, a model is proposed for the involvement of CKII-mediated phosphorylation in the regulation of Cx45.6 turnover and specific cleavage by caspase-3-like protease. In the outer cortex of the lens, when a full-length Cx45.6 is phosphorylated by CKII at Ser363, it becomes unstable and possibly undergoes degradation mediated through the proteasome pathway (17). The CKII phosphorylation site Ser363 is located within a PEST domain (17, 44), and this domain has been reported to be associated with a rapid protein turnover via proteasome-mediated degradation (45). Additionally, phosphorylation is also known to signal protein degradation by proteasome (46). When Ser363 is not phosphorylated by CKII, Cx45.6 renders itself into a substrate for caspase-3, which cleaves at Glu367 at the COOH terminus of Cx45.6 and generates truncated fragments of Cx45.6. These fragments were localized to the central core region of the lens.

We observed two fragments derived from Cx45.6 in the size of 46 and 48 kDa. These fragments were accumulated during lens development. The 46-kDa fragment was predominant during the embryonic stage, whereas the level of the 48-kDa fragment increased and reached a similar level as compared with the 46-kDa form at the postnatal stage. Formation of two fragments was also observed when GST-Cx45.6.6F1 was treated with caspase-3. However, mutation of a potential cleavage site in GST-Cx45.6F1 blocked the production of either fragment, suggesting that this site initiates generation of these two fragments. Because of the closeness of their migration, these two fragments sometimes were less distinctive on the SDS-PAGE. We also observed a potential third band larger than 48 kDa at P60 samples, and this band could be generated from additional cleavage or certain type of posttranslational modifications.

Our data suggest that caspase-3-like protease is responsible for the in vivo cleavage of Cx45.6. In contrast to our observation in chicken, a previous study has shown that a large portion of the COOH terminus of Cx50, the ovine ortholog of Cx45.6, is removed by calpain (32). This discrepancy could account for the
difference in expression level or accessibility of these two proteases expressed in these two species, because the responsibility of one protease could be replaced by the other during evolution. It is also likely that the cleaved fragment of Cx50 observed in ovine is caused by in vitro experimental manipulation. We found if lysates of lens primary cells were handled improperly, Cx45.6 would be cleaved into a 33-kDa fragment, an identical size fragment as the fragment cleaved by calpain (data not shown).

We have also shown that the truncated Cx45.6 was accumulated during lens development and was localized to the differentiated lens fibers. The activation of caspases-3 also initiates in the differentiated lens fibers and is involved in later stage cell differentiation, such as nucleus degeneration (18). Thus, the caspase-3-truncated Cx45.6 is likely to form a unique type of gap junctions, different from the ones formed by full-length Cx45.6. These intercellular channels may facilitate the passage of factors important for fiber differentiation and/or maintain the physiological functions of mature fibers. Functional gap junction channels are, indeed, formed by truncated Cx50 of lens consisting of truncated connexins may not be similarly inhibited by this chemical.

Lens Cx45.6 is the first connexin identified as a substrate for caspase. In addition to lens fibers other cell types, such as skin keratinocytes, also undergo degeneration of nuclei and organelles during cell differentiation. Caspase activation has been identified in the differentiation of epidermal keratinocytes and is required for the loss of the nucleus (49). It is of great interest to determine whether connexins in those cells are also subject to regulation by caspases. The physiological significance of the regulation of connexin by caspases requires further investigation.

Acknowledgments—We thank D. Adan-Rice and L. Wang for technical assistance and Z. Dong for valuable discussions. We thank members of the Jiang laboratory for critical reading of the manuscript.

REFERENCES
1. Rafferty, N., and Eason, E. (1974) J. Ultrastruct. Res. 46, 239–253
2. Mathias, R. T., and Rae, J. L. (1989) in Cell Interactions and Gap Junctions (Sperelakis, N., and Cole, W. C., eds) pp. 29–50, CRC Press, Inc., Boca Raton, FL
3. Cooper, K., Mathias, R. T., and Rae, J. L. (1991) in Biophysics of Gap Junction Channels (Perachon, C., ed) pp. 57–74, CRC Press, Inc., Boca Raton, FL
4. Musil, L. S., Cunningham, B. A., Edelman, G. M., and Goodenough, D. A. (1990) J. Cell Biol. 111, 2077–2088
5. Dermietzel, R., Traube, O., Hwang, T. K., Beyer, E., Bennett, M. V. L., Spray, D. C., and Willecke, K. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 10148–10152
6. Beardslee, M. A., Laing, J. G., Beyer, E. C., and Saffitz, J. E. (1998) Circ. Res. 83, 629–635
7. Fallon, R. F., and Goodenough, D. A. (1981) J. Cell Biol. 90, 521–526
8. Guan, X. J., and Ruch, R. J. (1996) Carcinogenesis 17, 1791–1798
9. Laing, J. G., and Beyer, E. C. (1995) J. Biol. Chem. 270, 26389–26403
10. Laing, J. G., Tadros, P. N., Westphale, E. M., and Beyer, E. C. (1997) Exp. Cell Res. 236, 482–492
11. Musil, L. S., Le, A.-C. N., VanSlyke, J. K., and Roberts, L. M. (2000) J. Biol. Chem. 275, 25257–25265
12. Berthoud, V. M., Bassnett, S., and Beyer, E. C. (1999) J. Cell Biol. 146, 247–257
13. Liu, Z., Galindo, R. L., and Wasserman, S. A. (1997) Genes Dev. 15, 3413–3422
14. Rechtesteiner, M., and Rogers, S. W. (1996) Trends Biochem. Sci. 21, 267–271
15. Langer, C. A., Shen, T., and Horvitz, R. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1032–1037
16. Le, A. N., and Musil, L. S. (1998) Dev. Biol. 204, 80–96
17. Guan, X. J., Wilson, S., Schlenker, K. K., and Ruch, R. J. (1996) Mol. Carcinog. 16, 157–164
18. Weil, M., Raff, M. C., and Braga, M. M. (1999) Curr. Biol. 9, 361–364

July 24, 2018 from http://www.jbc.org/ by guest

The Development-associated Cleavage of Lens Connexin 45.6 by Caspase-3-like Protease Is Regulated by Casein Kinase II-mediated Phosphorylation

Xinye Yin, Sumin Gu and Jean X. Jiang

J. Biol. Chem. 2001, 276:34567-34572.
doi: 10.1074/jbc.M106073200 originally published online July 11, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106073200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 21 of which can be accessed free at http://www.jbc.org/content/276/37/34567.full.html#ref-list-1