INTRODUCTION
The intervertebral disc has a hybrid collagen architecture that embodies features of ligament and cartilage in its structure and function. Nucleus pulposus, the gel-like central zone of the young intervertebral disc, has a similar collagen phenotype to that of hyaline cartilage, with types II, IX and XI collagens being the principal fibrillar components. In fetal cartilage, type XI collagen consists of molecules containing three genetically distinct chains, [1(XI)], [2(XI)] and [3(XI)] in a 1:1:1 ratio. The molecules are cross-linked by lysyl oxidase-mediated bonds, which exhibit distinct chain specificities (1). However, from mature articular cartilage, the isolated type XI collagen fraction includes a significant proportion of the [1(V)] chain (2), the chain ratios suggesting the existence of type V/type XI hybrid molecules in the tissue. It is possible that the type V/type XI hybrid molecules also exist in disc tissue. To test this possibility, we identified the molecular isoforms of type V/XI collagen present in the nucleus pulposus with a view to understanding how the type V/type XI chains are organized in the intervertebral disc matrix. Since collagen V/XI in general appears to act as a template in regulating the overall fibril architecture of a tissue, the unique organization of disc collagen may in part depend on a novel type V/XI phenotype.

MATERIALS AND METHODS
Nucleus pulposus was dissected from lumbar spines of 3-month-old calves. Tissue slices were extracted in 4M guanidine HCl, 0.05M Tris-HCl, pH 7.4 containing protease inhibitors, at 4°C for 24 h to remove proteoglycans and other matrix proteins, then washed thoroughly with water and freeze-dried. Cross-linked collagens were solubilized by digesting the washed residues with pepsin at 4°C. Pepsin digests were fractionated into collagen types II, XI and IX by precipitation at 0.7M, 1.2M and 2.0M NaCl, respectively. The individual type XI/V chains were then resolved by HPLC on a C4 reverse-phase column with a linear gradient (18-28%) of solvent B in A over 40 min followed by SDS-PAGE. Mass spectrometry was performed on a ThermoFinnnigan LCQ Deca XP with electrospray ionization source and in-line C8 RP-HPLC. Individual protein bands after Coomassie Blue staining on SDS-PAGE were digested in-gel by trypsin. The resulting peptides were subjected to tandem mass spectrometry. For protein identification, peptide fragments were compared with the FBSC non-redundant protein database using SEQUEST, an automated database search algorithm designed for use with tandem mass spectrometry data.

RESULTS AND DISCUSSION
Using the two dimentional HPLC/SDS-PAGE method, we are able to resolve all five type V/XI gene products, [1(V)], [2(V), [1(XI)], [2(XI)], and [3(XI)] chains, from each other. An unexpected pattern was found in the nucleus pulposus. Instead of the 1:1:1 ratio of [1(XI)]; [2(XI)]; [3(XI)] chains found in developing hyaline cartilage, two collagen V chains, [1(V)] and [2(V)], and [2(V)] chains were also prominent in nucleus, despite the tissue being collagen type II-based (Fig. 1). The chain identities, assigned from their elution on reverse-phase HPLC and migration on SDS-PAGE, were established beyond doubt by in-gel trypsin digestion, and microbore LC/mass spectrometry with data base matching (Fig. 2).

Collagen type V/XI gene products are best considered as members of the same collagen subclass. In cartilage, the [1(V)] chain becomes an integral component, increasing in proportion with increasing tissue maturity (2). Similarly in bone, the [1(XI)] chain accumulates with developmental age in the type V collagen fraction in which it is incorporated with [1(V)] and [2(V)] chains to form an [1(V)] [1(XI)] [2(V)] hybrid molecule (3). Hybrid molecules assembled from [1(XI)] and [2(V)] chains are also characteristic of bovine vitreous (4), an extracellular structure with similar physical properties to nucleus pulposus. Type I collagen is absent from nucleus pulposus, so the [1(V)] and [2(V)] chains are not simply due to a fibrocortilage collagen phenotype. Another difference between nucleus pulposus and hyaline cartilage is the expression of the short form of the [1(IX)] chain in nucleus, a product of an alternative promoter which lacks the NC4 globular domain (5). This short form of the [1(IX)] chain is also expressed in the vitreous but not hyaline cartilage (6). The present findings indicate that several heterotrimeric chain combinations are represented in the type V/XI collagen pool of the disc. The molecular forms and distribution pattern of the typeV/XI isoforms are likely to be related to the formation of distinctive fibril network of disc tissue.

REFERENCES