Differential Organization of Desmin and Vimentin in Muscle Is Due to Differences in Their Head Domains

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Abstract. In most myogenic systems, synthesis of the intermediate filament (IF) protein vimentin precedes the synthesis of the muscle-specific IF protein desmin. In the dorsal myotome of the *Xenopus* embryo, however, there is no preexisting vimentin filament system and desmin's initial organization is quite different from that seen in vimentin-containing myocytes (Cary and Klymkowsky, 1994. Differentiation. In press.). To determine whether the organization of IFs in the *Xenopus* myotome reflects features unique to *Xenopus* or is due to specific properties of desmin, we used the injection of plasmid DNA to drive the synthesis of vimentin or desmin in myotomal cells. At low levels of accumulation, exogenous vimentin and desmin both enter into the endogenous desmin system of the myotomal cell. At higher levels exogenous vimentin forms longitudinal IF systems similar to those seen in vimentin-expressing myogenic systems and massive IF bundles. Exogenous desmin, on the other hand, formed a reticular IF meshwork and non-filamentous aggregates. In embryonic epithelial cells, both vimentin and desmin formed extended IF networks. Vimentin and desmin differ most dramatically in their NH_2-terminal "head" regions. To determine whether the head region was responsible for the differences in the behavior of these two proteins, we constructed plasmids encoding chimeric proteins in which the head of one was attached to the body of the other. In muscle, the vimentin head-desmin body (VDD) polypeptide formed longitudinal IFs and massive IF bundles like vimentin. The desmin head-vimentin body (DVV) polypeptide, on the other hand, formed IF meshworks and non-filamentous structures like desmin. In embryonic epithelial cells DVV formed a discrete filament network while VDD did not. Based on the behavior of these chimeric proteins, we conclude that the head domains of vimentin and desmin are structurally distinct and not interchangeable, and that the head domain of desmin is largely responsible for desmin's muscle-specific behaviors.

DURING embryonic development most non-epithelial cell types transiently express the intermediate filament (IF)[1] protein vimentin before the synthesis of "terminal," cell-type specific IF proteins (Traub, 1985; van de Klundert et al., 1993). It seems likely that the differentiation-specific IF proteins, i.e., desmin, glial fibrillary acidic protein, peripherin, α-internexin, and the neurofilament proteins, are specialized for specific tasks in the cell or have properties compatible with the function of a particular cell type. For example, it is now clear that a specific role of the neurofilament proteins is to maintain axonal caliber in certain types of neurons (Hoffmann et al., 1985, 1988; Ohara et al., 1993; Eyer and Peterson, 1994). It also appears that the ectopic expression of IF proteins can lead to cellular defects (Capetanaki et al., 1989; Dunia et al., 1990; Blessing et al., 1993; Côté et al., 1993; Xu et al., 1993). Presumably different IF proteins have different affinities for intracellular structures or different mechanical properties that make a particular IF well suited for one cell type but poorly suited for another.

Muscle provides a striking example of how a change in composition of IFs correlates with changing cellular organization. In most vertebrate systems examined, muscle precursor cells initially synthesize vimentin. As myogenic differentiation begins, these cells initiate synthesis of the muscle-specific IF protein desmin (Bennett et al., 1979; Gard and Lazarides, 1980; Holtzer et al., 1982). Vimentin and desmin readily coassemble with one another both in vitro (Steinert et al., 1981) and in vivo (Quinlan and Franke, 1982; Tokuyasu et al., 1985). The newly synthesized desmin integrates into the preexisting vimentin filament network, thus desmin's initial distribution in the cell reflects that of vimentin. As myogenic differentiation proceeds, the relative levels of vimentin and desmin change: vimentin synthesis ceases and desmin becomes the major, and in most cases, the only IF protein in the mature muscle cell (Bennett el al.,

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1. *Abbreviations used in this paper*: DVV, desmin head and vimentin-rod tail; IF, intermediate filament; VDD, vimentin head and desmin-rod tail.

1979; Granger and Lazarides, 1979; Gard and Lazarides, 1980; Holtzer et al., 1982). This change in IF composition is accompanied by a rather dramatic change in IF organization: the originally longitudinal IF system is transformed into a transverse system associated with the Z-lines, the sarcolemma, and the myotendinous junction (Bennett et al., 1979; Granger and Lazarides, 1979; Craig and Pardo, 1983; Tokuyasu et al., 1985; Tidball, 1992). This membrane-associated, transverse IF network has been implicated in the production of restorative forces after muscle contraction (Brady, 1984) and in the general mechanical integration of the muscle cell (Lazarides, 1980).

The reorganization of the muscle IF system occurs simultaneously with the change in IF composition. It is not clear whether vimentin and desmin differ with respect to either network formation or mechanical properties or whether the change in IF composition influences IF organization. One system that can be used to address these questions is the dorsal myotome of the *Xenopus* embryo/tadpole. In this muscle, vimentin is never expressed (Dent et al., 1989; Herrmann et al., 1989*a*) and the desmin filament system forms de novo (Hermann et al., 1989*b*; Cary and Klymkowsky, 1994). In fact, the pattern of desmin organization during *Xenopus* dorsal myotome differentiation differs markedly from that described in systems where vimentin is present (Cary and Klymkowsky, 1994). Desmin fails to form the longitudinal IF system characteristic of vimentin-containing myotubes. Rather, it is initially concentrated at the intersomite junction and the lateral sarcolemma. As myogenic differentiation proceeds, a reticular network of desmin filaments appears followed by the association of desmin with Z-lines.

The differences between the *Xenopus* dorsal myotome and other myogenic systems suggests that vimentin and desmin differ significantly in the types of IF networks they form *in vivo*. To study this question further, we have examined the behavior of epitope-tagged exogenous vimentin and desmin proteins in embryonic cells. Myocytes expressing the exogenous vimentin contained longitudinal IF systems. On the other hand, expression of an identically tagged form of desmin resulted in the formation of reticular desmin IFs and non-filamentous aggregates. To determine the regions of these proteins involved in determining their behavior, we constructed plasmids encoding chimeric proteins. These experiments point to a role for the amino-terminal head domain in the differential organization of vimentin and desmin in muscle. They also indicate that the head domains of these two IF proteins are not structurally equivalent.

Materials and Methods

Construction of Epitope-tagged Vimentin and Desmin Expression Plasmids

cDNAs encoding either the *X. laevis* vimentin-1 (Dent et al., 1992) or desmin (Herrmann et al., 1989b) proteins were amplified by PCR (95°C for 3', 5 cycles of 95°C for 2', 37°C for 2', 72°C for 2' followed by two cycles of 95°C for 2', 72°C for 2' and then 72°C for 8') to add restriction sites. PCR products were subcloned into the pSK.tag plasmid (Dent et al., 1992) using *Nde I* and *Xba I*. This adds the SRDSTMEQKLISEEDLN.stop encoding sequence, recognized by the monoclonal antibody 9E10, "in-frame" to the COOH terminus of the inserted protein coding sequence (Fig. 1 A). To express the proteins in *Xenopus* embryos, the tagged IF protein coding sequences were subcloned into pCskAct (Fig. 1 B). pCskAct, provided by Richard Harland (UC Berkeley), contains the *X. borealis* cytoskeletal actin promoter and the SV40 polyadenylation signal (Cross et al., 1988; Smith and Harland, 1991). The vimentin-1.tag and desmin.tag sequences were also subcloned into the mammalian expression vector pSRα (Takebe et al., 1988).

Construction of Head/Body Chimeras

Oligonucleotide primers were designed to generate "seam-less" fusions between the desmin head and vimentin-rod/tail (DVV) and the vimentin head and desmin-rod/tail (VDD) (Fig. 1 C). The upstream primer used to amplify the desmin head added an *Nde I* site (5' CCCCATATGAGCCAGTCCTATTCAAG3') to the 5' end of the coding sequence while the downstream primer added *Xho I* (dotted underline) and *Xba I* (solid underline) sites (5' CCCTCTAGAGAACTCGAGAACATCTGCTCCATAGCTGC 3'). The vimentin body was amplified using an upstream primer which added *Nde I* (solid underline) and *Sal I* (dotted underline) sites (5' CCCCATATGGTCGACTTCGCCCTGGCAGATGCC 3') and a downstream primer that added an *Xba I* site (5' CCCTCTAGACTCAAAGTCATCGTGGTGCTG 3'). PCR products were subcloned independently into a *Xho I* free version of pSK.tag using *Nde I* and *Xba I*. Restriction of pSK.desminHead.tag with *Xho I* and *Xba I* generated a site into which the *Sal I/Xba I* restricted vimentin rod/tail domain could be ligated, *Sal I* and *Xho I* overhangs are compatible but the resulting junction can not be restricted with either enzyme. To construct VDD we used a similar strategy but placed the *Xho I* (underlined) site on the desmin body (5' CCCCATATGCTCGAGTTCAGTCTTGCAGATCGAATGAAC 3') while adding the *Sal I* (underlined) site to the vimentin head (5' CCCTCTAGAGAAGTCGACGGAGTCTGCCATTCTGGCCGG 3'). The final products were sequenced to confirm the junction between the two regions. In the original construction of the vimentin head, desmin body chimera a single amino acid was changed from Asp to Glu. To revert this mutation the PCR based procedure of Hemsley et al. (1989) was used. Reversion of the mutation generates a *Sal I* site at the junction between head and body domains, which was used in the original identification of revertant plasmids. The final plasmid was sequenced and then subcloned into pCskAct. The sequences at the head/rod junction are shown in Fig. 1 C.

DNA Injection into Cultured Cells

To synthesize proteins in cultured cells, supercoiled plasmid DNA was injected into nuclei of either human SW13 clone 2 cells (Sarria et al., 1990) (supplied by R. M. Evans - UC Health Sciences Center, Denver) or *Xenopus* A6 cells (obtained from Amer. Type Culture Collection, Rockville, MD) using a simple manual pressure-driven injection system (Klymkowsky, 1981). SW13 clone 2 cells were cultured in 10% FCS, 50 μg/ml gentamycin in minimal Eagle's medium; A6 cells were cultured in Leibowitz L-15 media supplemented with 10% FCS and gentamycin at 16°C. Injected cells were fixed 2–20 h after injection with 100% methanol and stained with the 9E10 antibody and fluorescein-conjugated goat anti-mouse immunoglobulin antibody (antiMIgFl)(Sigma Chem. Co., St. Louis, MO) to visualize the exogenous proteins. The 9E10 hybridoma line can be obtained from the American Type Culture Collection.

DNA Injection into Fertilized Eggs

Xenopus eggs were fertilized after standard procedures and injected at the 1-cell stage with *Sca I* linearized plasmid DNA using the same basic method used for the injection of antibodies (Klymkowsky et al., 1992). The linearized DNA was isolated from a 1% agarose gel, purified using glass beads (Vogelstein and Gillespie, 1979) and eluted in water; it was injected at a concentration of ~50 μg/ml. Each egg received ~10 nl of DNA solution. In this study, injected embryos were harvested for whole-mount immunocytochemistry at stage 38. Myocytes were identified based on their distinctive morphology and position within the embryo.

Whole-mount Immunocytochemistry

To visualize the distribution of exogenous proteins, plasmid DNA-injected embryos were fixed, bleached, and stained in whole-mount (Dent et al., 1989; Klymkowsky and Hanken, 1991) using the 9E10 antibody (5–10 μg/ml). Bound antibody was visualized by staining with either horseradish peroxidase–conjugated goat anti-mouse immunoglobulin antibody (antiMIgPO) (Biorad Labs.) or by staining with antiMIgFl. Peroxidase-stained embryos were reacted with diaminobenzidine, cleared, and examined using a Zeiss IM35 microscope. Fluorescein-stained embryos were cleared and examined using a Laser Scanning Confocal microscope (Molecular Dynamics, Inc., Sunnyvale, CA) (five scans averaged).

Light and Electron Microscopic Section Analyses

For immunoelectron microscopy embryos were fixed in 4% paraformaldehyde, 0.1% glutaraldehyde, 10 mM $MgCl_2$, 10 mM $CaCl_2$ 150 mM sodium cacodylate (pH 7.4) overnight at 4°C. They were then rinsed for 30 min in 150 mM glycine, 150 mM sodium cacodylate (pH 7.4) at room temperature, and then dehydrated in an ethanol series. Dehydrated embryos were infiltrated with LR White resin (20% resin for 1 h; 50% resin for 2 h; 66% resin for 4 h; and then 100% resin overnight at 4°C). The next day the resin was replaced and the embryos were rocked at room temperature for 6 h; again the resin was replaced and the embryos were transferred to gelatin capsules and placed in a vacuum oven for 36 h at 58°C. After polymerization blocks were trimmed and sections were cut on a dry glass knife; the 2.5 μm thick sections were transferred to a drop of water on a polylysine-coated coverslip warmed to 48°C. Dried sections were then stained for 1 h at room temperature in a humid chamber with 50 μg/ml 9E10 antibody followed by antiMIgFl. Antibodies were diluted into blocking solution (blocking solution: phosphate-buffered saline containing 0.8% BSA, 0.02% Tween-80 and 0.1% fish gelatin [Amersham Corp., Arlington Heights, IL]). Sections were mounted and examined; those sections that contained 9E10-stained cells were photographed and transferred to coverslips then glued to Epon blocks using cyanoacrylate-based glue (Satellite city "Hot Stuff"™ brand). Blocks were trimmed and sectioned; the 70–80 nm thin-sections were placed on formvar-coated nickel slot grids. For immuno-gold staining, grids were placed in blocking solution for 15 min to reduce non-specific antibody binding, and then in 2 μg/ml 9E10 antibody in blocking solution. Grids were rinsed briefly in blocking solution, and then incubated with 10 nm gold-conjugated anti-mouse antibody (Amersham Corp.) diluted 1:10 in blocking solution. All incubation were carried out in a humid chamber at room temperature for 1 h. After rinsing in PBS grids were fixed in 1% glutaraldehyde in PBS, rinsed in distilled water and counter stained with heavy metals; 2% uranyl acetate for 8 min followed by Reynold's lead citrate for 3 min.

Results

In *X. laevis* vimentin is not normally present in the cells of the dorsal myotome. To determine whether exogenous vimentin behaves like the intersomite junction/plasma membrane-associated endogenous desmin system (Cary and Klymkowsky, 1994), or more like the longitudinal vimentin filaments characteristic of other myogenic systems (Bennett

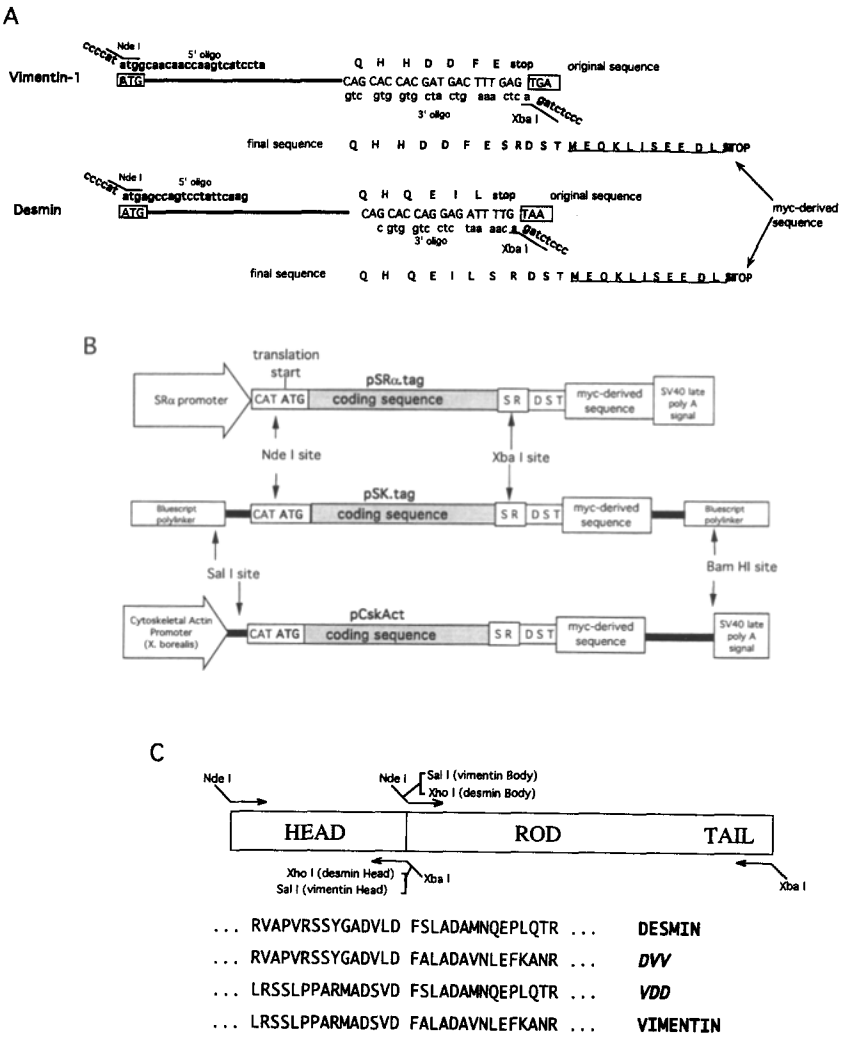

Figure 1. (*A*) Oligonucleotides were used to amplify the coding regions of the *Xenopus* vimentin-1 and desmin cDNAs. These sequences were then subcloned into the pSK.tag plasmid using the Nde I and Xba I sites introduced during amplification. This links the coding region for the IFP to the human c-*myc* derived tagging sequence. The sequence of the junction is shown. (*B*) Once subcloned into pSK.tag, the IF coding sequence and in frame tag coding sequence was subcloned into either pSRα or pCskAct. Sal I/Bam HI digestion was used for subcloning into pCskAct; Nde I/Xba I digestion was used for subcloning into pSRα.tag. (*C*) For the construction of chimeric coding sequences Nde I and Xba I sites were introduced to the 5′ and 3′ ends of the sequences encoding the domains of interest. This allowed PCR products to be subcloned into a Xho I minus version of pSK.tag. Next the pSK.vimentin-Body.tag plasmid was restricted with Xho I and Xba I and the body insert ligated into the Sal I/Xba I restricted pSKdesminHead.tag plasmid. Similarly, the pSKdesminBody.tag plasmid was restricted with Sal I and Xba I and the body insert ligated into the Xho I/Xba I restricted pSKvimentinHead.tag plasmid. The resulting VDD construct carried an asp to glu mutation at the head-body junction. PCR based site directed mutagenesis (Hemsley et al., 1989) was used to revert this mutation. From pSK.tag, sequences encoding tagged chimeric proteins were excised using Sal I and Bam HI and subcloned into pCskAct (see Fig. 1 *B*). The final sequence of the head/rod junctions of junctions of DVV and VDD are shown here. Junctions are "seam-less" in that no amino acids have been introduced or deleted from either parent molecule.

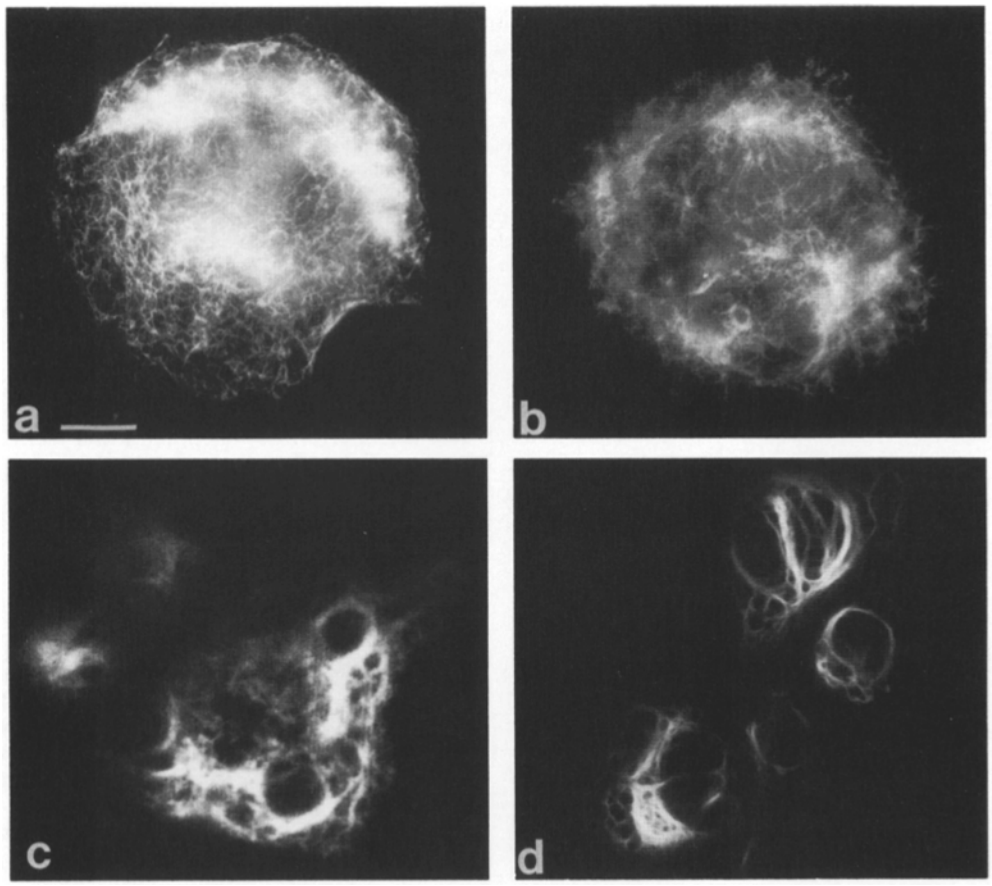

Figure 2. Tagged desmin and vimentin form IF networks. We injected supercoiled pSR-α.desmin.tag (*a*) or pSR-α.vimentin-1.tag (*b*) DNA into the nuclei of the IF-free human adenocarcinoma cell line SW13 clone 2. After 2 h both desmin and vimentin had assembled into extensive filament systems as visualized by staining with the anti-tag antibody 9E10 and fluorescein-conjugated secondary antibody. 9E10 does not stain uninjected cells (data not shown). Similarly, when fertilized eggs were injected with pCskAct.desmin.tag (*c*) or pCskAct.vimentin-1.tag DNA (*d*), both proteins were found to assemble IF networks in embryonic epithelial cells, these cells contain a keratin IF system but no type III IFs. Bar in *a* marks 10-μm for all parts.

et al., 1979; Granger and Lazarides, 1979; Gard and Lazarides, 1980; Holtzer et al., 1982), we subcloned the *X. laevis* vimentin-1 and desmin coding sequences into the *Xenopus* expression vector pCskAct or the mammalian cell expression vector pSRα (Fig. 1 *b*). To distinguish the exogenous proteins from the endogenous forms both vimentin-1 and desmin coding sequences were modified to place an epitope-tag at the COOH terminus of the encoded protein (Fig. 1 *a*). The tagging sequence, derived from the human *c-myc* protein, is recognized by the monoclonal antibody 9E10 and appears to have little if any effect on the ability of tagged IF proteins to form filaments in vivo (Christian et al., 1990; Gill et al., 1990; Wong and Cleveland, 1990; Dent et al., 1992; Bachant, 1993).

In our experiments, supercoiled pSRα DNA was injected into the nucleus of cultured cells; this led to the appearance of encoded polypeptide in a high percentage of the cells within 1–4 h of injection. Linearized pCskAct DNA was injected into fertilized *Xenopus* eggs; this leads to the expression of the encoded protein in a wide-range of cell types in the later stage embryo (Vice et al., 1991). In embryonic cells, the level to which exogenous polypeptides accumulate can vary dramatically. The reason(s) for these differences are unclear. It is relatively straightforward, however, to quantify the level of accumulated protein (low, moderate, or high) because each of the proteins used in this study carried the same epitope at the same position in the polypeptide (Fig. 1 *a*). Since all polypeptides were visualized using the same primary and secondary antibodies, the expression levels could be compared between cells and embryos by keeping laser power, photomultiplier tube voltage, and dark level settings constant; similarly, for peroxidase-stained whole-mounts, the time of the DAB-reaction was also kept constant.

We first examined the ability of the myc-tagged forms of vimentin-1 and desmin to form filament networks in both the IF-minus human cell line SW13.clone 2 (Fig. 2, *a* and *b*) (Sarria et al., 1990, 1992) and in embryonic epithelial cells (Fig. 2, *c* and *d*), which express only keratin-type IF proteins (Dent et al., 1989; Herrmann et al 1989*a*,*b*). In both cases, the myc-tagged vimentin and desmin proteins formed IF networks. There is a difference, however, between the quality of these networks in the two systems. In SW13.clone 2 cells both proteins formed fine, discrete filament networks (Fig. 2, *a* and *b*), whereas in embryonic epithelial cells (Fig. 2, *c* and *d*) large filament cables were formed. This difference could reflect temperature effects (Herrmann et al., 1993), since *Xenopus* embyros are maintained at 16°C whereas SW13 cells are maintained at 37°C. Alternatively, cellular factors could be influencing IF organization (see Dent et al., 1992).

Behavior of Exogenous Vimentin and Desmin in the Myotome

In myotomal myocytes of the stage 38–40 tadpole endogenous desmin forms a sparse, reticular meshwork that is associated with the sarcolemma and the newly forming Z-discs (Cary and Klymkowsky, 1994). Some myocytes also display desmin aggregates (Cary and Klymkowsky, 1994). The be-

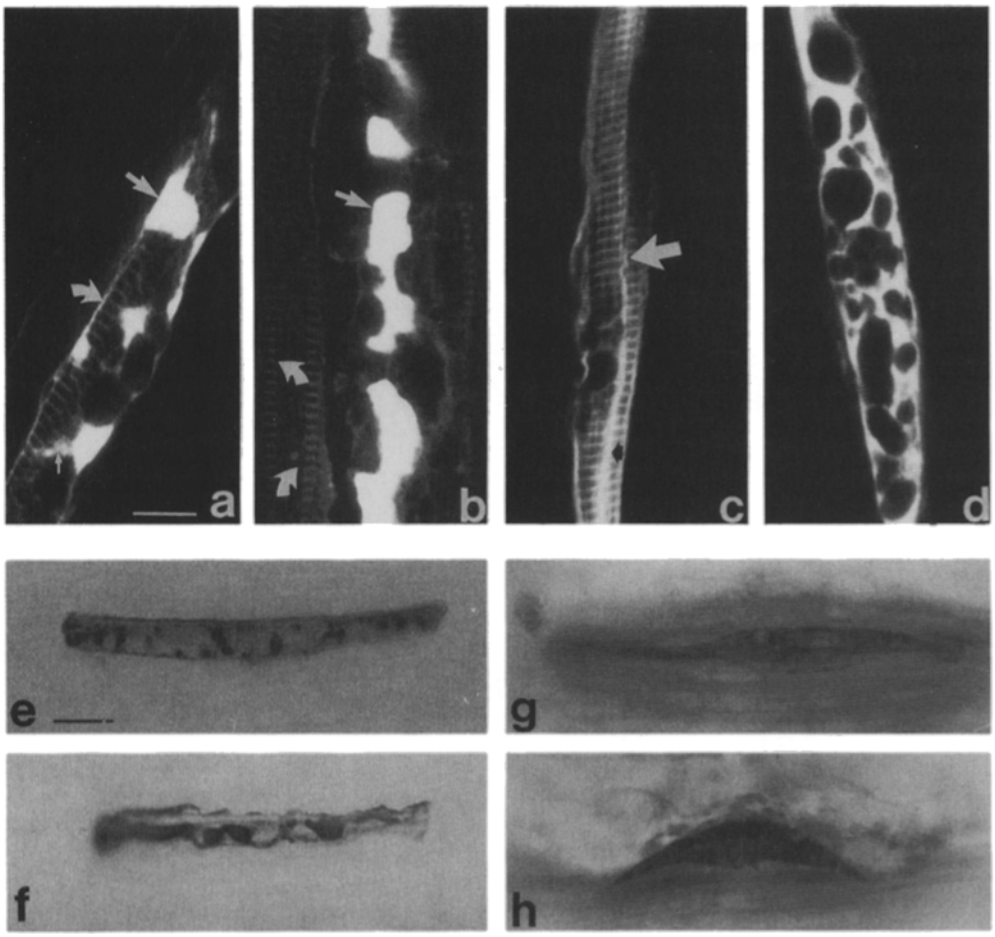

Figure 3. Behavior of exogenous desmin and vimentin-1 in muscle. Fertilized eggs were injected with linear pCskAct.desmin.tag or pCskAct.vimentin-1.tag DNA and fixed at stage 38/40. Embryos were stained in whole-mount using 9E10 and fluorescein-conjugated secondary antibody. At moderate levels of accumulation the exogenous desmin associated with the sarcolemma (*curved arrow* in *a*), Z-lines and formed both a fine filament meshwork (*small arrow*) and non-filamentous aggregates (*large arrow*). The aggregates are a prominent component of the sarcoplasm in cells accumulating higher levels of the protein (*straight arrow, b*); meshwork, sarcolemmal, and Z-line staining are also present in such cells but fall out of the plane optical section shown. At low levels of desmin accumulation the protein is associated with Z-lines (*curved arrows, b*) and the sarcolemma (not shown). Myocytes accumulating moderate levels of exogenous vimentin contain longitudinal filaments and filament bundles (*arrows* in *c*) containing the exogenous protein. At higher levels of accumulation the exogenous vimentin forms massive inclusions that often occupy large portions of the sarcoplasm (*d*). To visualize the overall morphology of cells, embryos were stained in whole-mount with 9E10 and peroxidase-conjugated secondary. Myocytes accumulating low to moderate levels of exogenous desmin (*e*) or vimentin (not shown) had a normal rectangular morphology. Accumulation of high levels of exogenous desmin was commonly associated with a crumpled or wrinkled appearance (*f*). High level accumulation of exogenous vimentin-1 appears to distort cell shape (*g* and *h*). Bar in *a* marks 10 μm for *a–d*. Bar in *e* marks 30 μm for *e–h*.

havior of exogenous desmin was similar to that of endogenous desmin. At low levels of accumulation, the exogenous desmin was found to incorporate into the endogenous desmin system and was primarily associated with Z-lines and the sarcolemma (Fig. 3, *a* and *b*). At higher levels of protein accumulation, many, if not all, of the myocytes expressing exogenous desmin also contained discrete aggregates of exogenous desmin (Fig. 3, *a*, *b*, and *e*). No extensive longitudinal filament network was formed by exogenous desmin at any level of accumulation. Ultrastructural analysis of myocytes containing the exogenous desmin protein revealed the presence of sharply delimited, 9E10-reactive aggregates scattered throughout the sarcoplasm, often localized near the sarcolemma or the nucleus (Fig. 5 *a*). These desmin aggregates appeared to be composed of both granular and filamentous material (Fig. 5 *b*); 10 nm filaments were associated with the periphery of some aggregates.

At low levels of accumulation, exogenous vimentin also integrated into the endogenous desmin system (Figs. 3 *c* and 4 *a*). In contrast to desmin, however, exogenous vimentin was also found to form longitudinal filamentous arrays when present at moderate levels (Figs. 3 *c* and 4 *a*). At higher levels of protein accumulation large, dense, irregular inclusions were found (Figs. 3 *d* and 4 *a*). These vimentin inclusions often occupied a large portion of the cell displacing cytoplasmic components (Figs. 3 *d* and 5 *c*). Ultrastructural analysis revealed these vimentin inclusions to be composed of bundles of 9E10-reactive 10-nm filaments (Fig. 5 *d*).

Effects of Overexpressing Exogenous Proteins on the Myocyte

Comparing the signals from whole-mount in situ hybridization analysis of endogenous desmin to those of exogenous desmin and vimentin, it is clear that the level of expression of the exogenous DNA can be many times greater than that of the endogenous gene (data not shown). In myocytes accumulating such high levels of exogenous vimentin or desmin, the normal roughly rectangular morphology of the myocytes was severely effected (Fig. 3, *f–h*). Cells expressing high levels of vimentin-1 commonly appeared spindle (Fig. 3 *g*) or club shaped (Fig. 3 *h*) while cells accumulating large amounts of exogenous desmin appeared wrinkled (Fig. 3 *f*) and sometimes detached from the intersomite junction (myo-

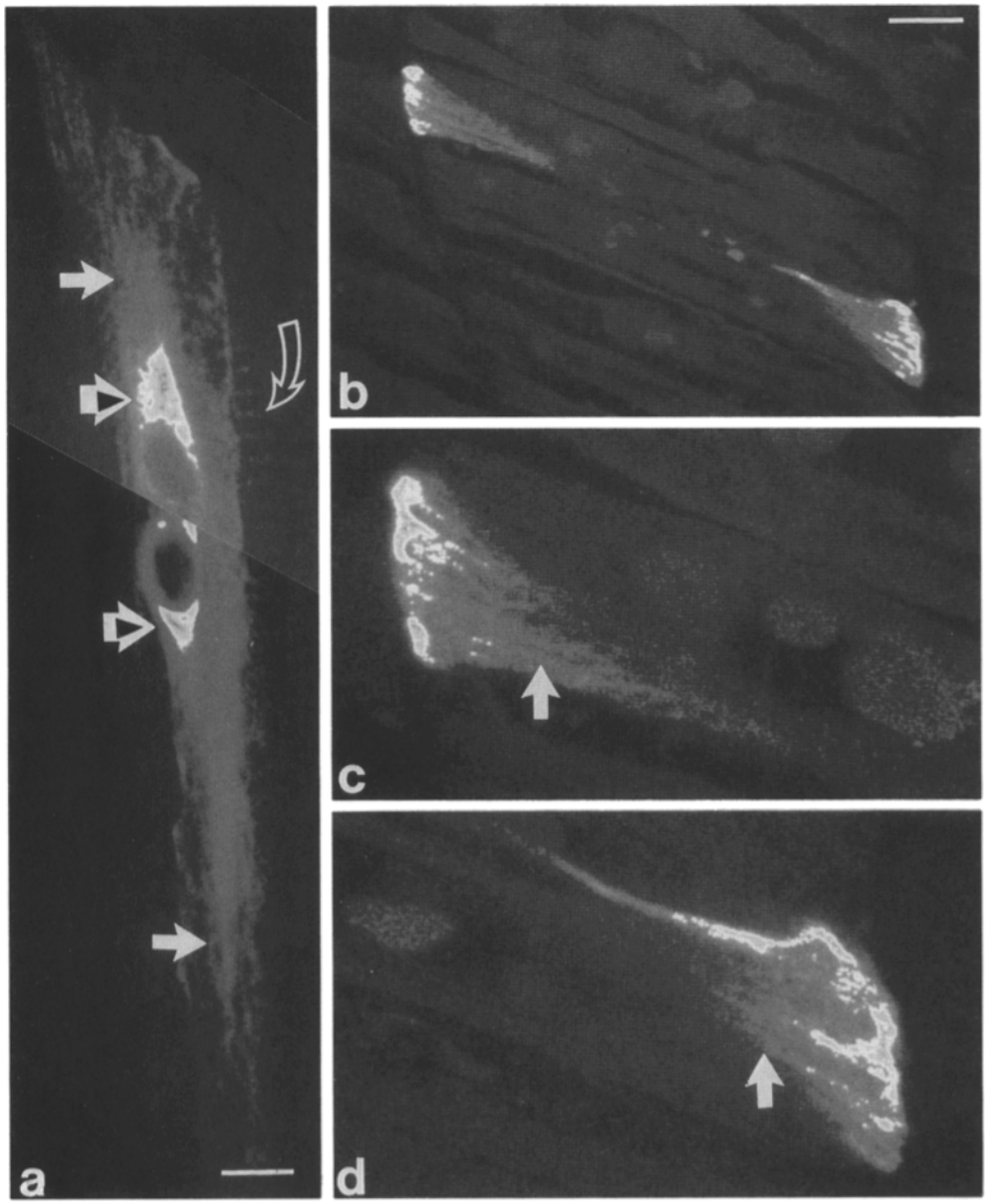

Figure 4. The longitudinal system formed by vimentin and VDD. Myocytes expressing exogenous vimentin (*a*) or VDD (*b–d*) were stained in whole-mount using 9E10 and fluorescein-conjugated secondary antibody and examined using confocal microscopy. (*a*) A longitudinal filament system (marked by *white arrows*) is present in this cell, which has accumulated a moderate level of exogenous vimentin. In this same cell are dense accumulations of vimentin (*black on white arrows*). In a neighboring cell expressing exogenous vimentin at low level (marked by *curved, open arrow*), the vimentin is associated exclusively with Z-lines. (*b*) At low power, a cell expressing moderate levels of VDD protein shows a longitudinal filament system associated with the ends of the myocyte. *c* and *d* show the two ends of this cell at higher magnification. Longitudinal filaments are pointed out by *white arrows*. Bar in *a* marks 5 μm for *a*, *c*, and *d*; Bar in *b* marks 10 μm.

septum) (not shown). In cells expressing lower levels of exogenous vimentin or desmin, cellular morphology appeared normal (Fig. 3 *e*).

Why Do Vimentin and Desmin Behave Differently?

Sequence comparison indicates that the *X. laevis* vimentin-1 and desmin proteins differ primarily in their NH_2-terminal head domains (Fig. 6). To test whether the difference in the behavior of vimentin and desmin was due to differences in their head domains, we constructed plasmids encoding chimeric proteins in which the head of one protein was fused to the body (rod and tail) of the other in a "seam-less" manner, i.e., without the deletion or addition of amino acids (Fig. 1 *C*). We chose the site of the junction to be the first highly conserved amino acid of the rod domain, Asp_{78} (in both proteins). The vimentin head/desmin rod and tail construct is referred to as VDD, similarly the complementary chimera composed of the desmin head/vimentin rod and tail domains is referred to as DVV. The coding regions of the chimeric proteins were subcloned into pSK.tag to add the *c-myc* based tagging sequence, and then subcloned into the pCskAct plasmid for expression in *Xenopus* embryos and cultured cells (Fig. 1, *B* and *C*).

In A6 cells both DVV and VDD proteins accumulated and incorporated into the endogenous vimentin filament system without apparent effects on vimentin filament organization (data not shown). When synthesized in embryonic epithelia cells, however, the two chimeric proteins behaved very differently. In epithelia cells expressing DVV, the protein formed discrete filament networks in the majority of cells examined (Fig. 7 *a*). VDD, on the other hand, formed few discernible filaments. The majority of the exogenous VDD protein was confined to large inclusions (Fig. 7 *b*). In stage 38 embryonic myocytes DVV formed a subsarcolemmal system, filament meshworks and cytoplasmic aggregates (Fig. 7, *c* and *d*), indistinguishable from those formed by exoge-

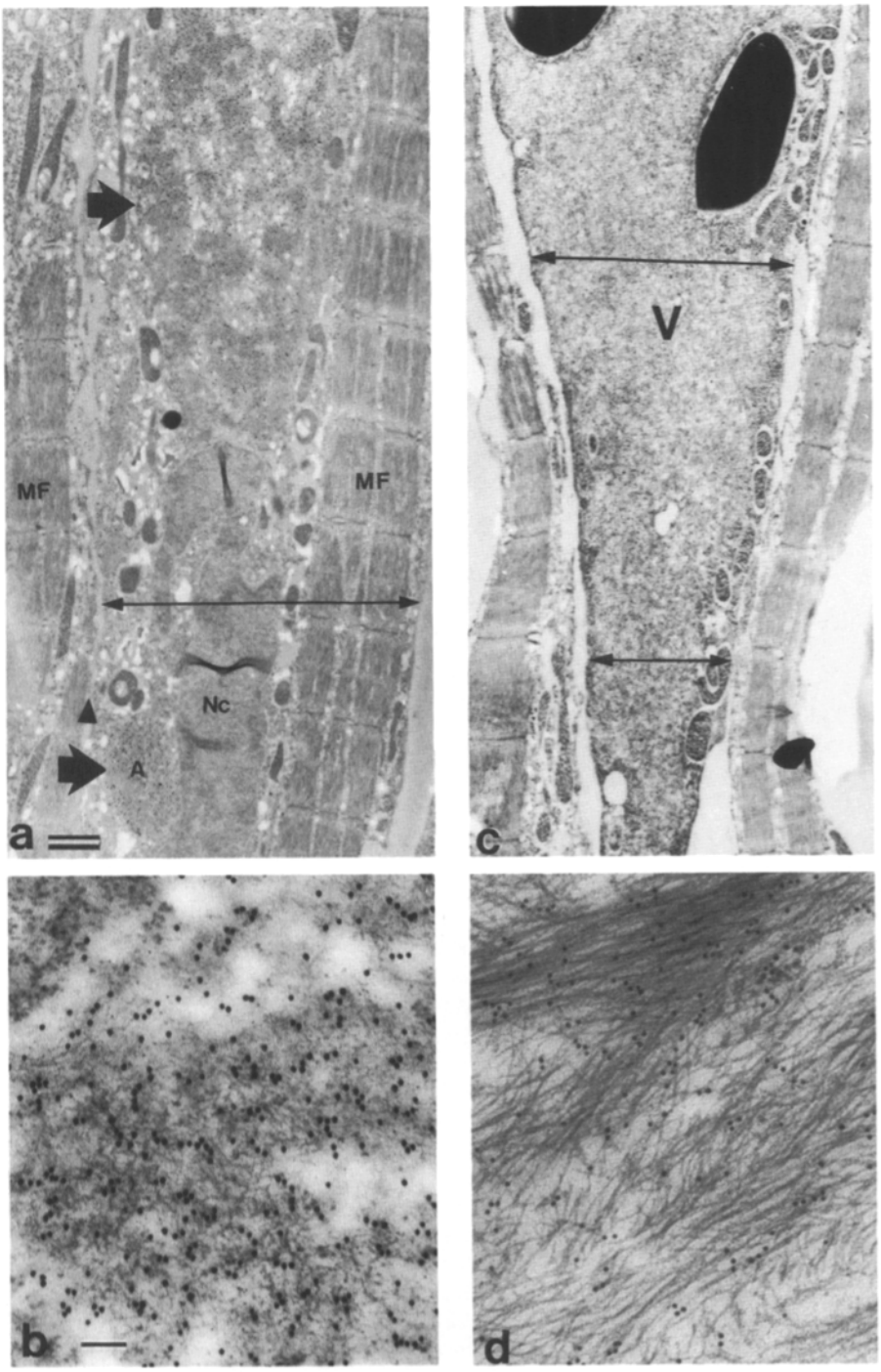

Figure 5. Ultrastructural analysis of exogenous desmin and vimentin. Myocytes expressing moderate to high levels of exogenous desmin (*a–b*) or vimentin (*c–d*) were examined using immunogold electron microscopy. (*a*) At low magnification, discrete aggregates of desmin can be seen (*arrows*) (*MF*, myofibrils; *Nc*, nucleus; *double-headed arrow* marks the myocyte expressing exogenous desmin; a cell not expressing the exogenous protein is to the left). (*b*) A higher magnification micrograph of a desmin aggregate composed of nonfilamentous material. (*c*) Vimentin, in contrast, forms filament bundles (*V*) that spread through large areas of the myocyte. The cell shown here displays an abnormal spindle shape, visible here as a dramatic tapering of the cell (*double-headed arrows*), and mitochondria appear displaced to the cell periphery. (*d*) At higher magnification the filamentous organization of exogenous vimentin-1 can be seen. Bar in *a* marks 1 μm for *a* and *c*. Bar in *b* marks 100 nm for *b* and *d*.

nous desmin (see above – Fig. 3, *a* and *b*). In contrast, VDD's behavior in myocytes was identical to that of exogenous vimentin-1. Myocytes synthesizing VDD contained longitudinal filaments (Figs. 4, *b–d* and 7 *e*) and large inclusions (Fig. 7 *f*). Ultrastructural examination of VDD and DVV-containing myocytes revealed that the DVV aggregates (Fig. 8 *a*) were similar to the non-filamentous aggregates seen in cells expressing exogenous desmin (Fig. 5 *b*), whereas the VDD inclusions were composed of discrete 10-nm filaments (Fig. 8 *b*), identical to the filaments seen in vimentin-expressing myocytes (Fig. 5 *d*). Based on these observations it seems that the head domains of vimentin and desmin are not interchangeable and that it is these head domains that largely determine the organizational behavior of the proteins in vivo. The behavior of the chimeras also indicates that the tagging epitope does not significantly influence the differential behavior of vimentin and desmin.

Discussion

Skeletal muscle development presents a dramatic example of IF network reorganization. In mammalian and avian muscle, myoblasts contain vimentin-type IFs. These myoblasts fuse with one another to form the syncytial myotube. Upon syn-

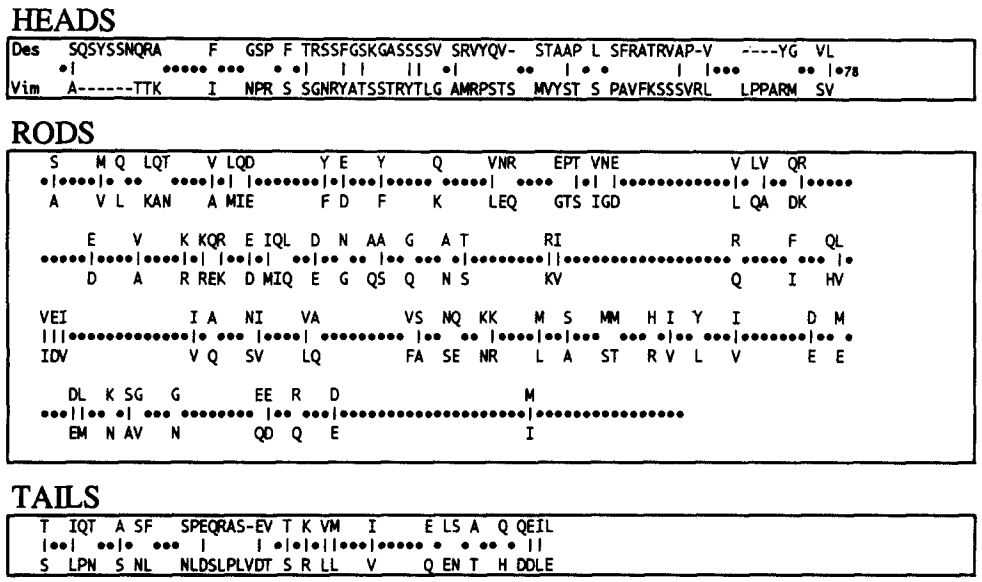

Figure 6. Sequence comparison between Xenopus vimentin-1 and desmin. The sequences of *Xenopus* desmin (Herrmann et al., 1989*a*) and vimentin-1 (Herrmann et al., 1989*b*) are compared to illustrate the relative lack of homology between their head domains and the substantial homology between their rod and tail domains. Identical residues are marked with a solid circle and conservative changes with an "*I*"; for both conservative and non-conservative changes, residues in both sequences are indicated using the single letter code. For the purposes of this analysis the following residues are considered conservative substitutions for one another: *R* and *K*; *D* and *E*; *S*, *T*, and *Y*; *S* and *A*; *Y* and *F*; *V*, *I*, *L*, *A*, and *M*; and *N* and *Q*. Spaces introduced into the sequence for the purpose of alignment are indicated by a "-". Amino acid 78, the site at which head and body domains are swapped in the chimera proteins, is indicated.

thesis, desmin integrates into the myotube's preexisting vimentin filament system. Vimentin therefore acts as a scaffold for the organization of desmin during the early phase of myogenesis. As myogenesis progresses, the initially longitudinal array of vimentin/desmin filaments is transformed into a transverse system that associates with the sarcolemma and the Z-discs of myofibrils. Concurrent with this change in IF organization there is a change in the composition of the IF system from a vimentin-dominated network to a system composed predominantly of desmin. Other changes to the cell's IF system also occur during this period, e.g., the intermediate filament-associated protein IFAPa-400 disappears (Cossette and Vincent, 1991). It is therefore difficult to know whether the changes in the vimentin/desmin ratio itself has effects on IF organization. Experimental study of this point has been complicated by the lack of vimentin-free myogenic systems.

It is in this light that the *Xenopus* dorsal myotome represents a unique opportunity for the study of IF organization. Vimentin is not expressed in the myotomal myocytes of *Xenopus* (Dent et al., 1989; Herrmann et al., 1989*a*). Transient expression of the IF protein nestin has been reported during rat myogenesis (Sejersen and Lendahl, 1993), but it is not known whether nestin is expressed in the *Xenopus* myotome. In any case, it would appear that the IF system of the early *Xenopus* myotome is composed primarily of desmin, which makes it possible to study the behavior of desmin in the absence of a preexisting vimentin filament system. A simple description of the distribution of desmin in the dorsal myotome (Cary and Klymkowsky, 1994) indicates that, in fact, desmin's organization in the absence of vimentin is quite different from that seen in vimentin/desmin-containing systems. No longitudinal IF system is formed; rather, desmin is associated with the sarcolemma in general and the intersomite junction in particular. As myogenesis proceeds the submembranous desmin system invades the sarcoplasm largely by association with myofibrils at the level of the Z-line; during this period, only a sparse, reticular cytoplasmic desmin filament network is present.

One could argue that the difference in IF organization in *Xenopus* myotomal muscle, compared to that seen in other myogenic systems, was due to *Xenopus*-specific factors. To examine this possibility, we forced the ectopic expression of vimentin in cells of the developing myotome. Although it is difficult to precisely quantify the level of exogenous protein in each cell, it is possible to accurately distinguish between cells expressing low, medium, and high levels of the exogenous proteins. There does not appear to be any significant difference in the levels to which exogenous vimentin, desmin, DVV, or VDD polypeptides accumulate within myotomal myocytes. Cells containing low, medium, or high levels of exogenous polypeptide were found for all four constructs. Because all four constructs are tagged in the same manner and recognized using the same antibodies, it appears likely that their levels of accumulation in myotomal myocytes are similar.

At low levels of accumulation both exogenous desmin and vimentin integrated into the endogenous desmin system (Figs. 3 *b* and 4 *a*). At moderate levels of expression, exogenous vimentin was found to form longitudinal filaments in myotomal myocytes (Figs. 3 *c* and 4 *a*); these longitudinal filaments are quite different from the filaments formed by the endogenous desmin system (Cary and Klymkowsky, 1994) or by exogenous desmin (Fig. 3, *a* and *b*). At higher levels exogenous vimentin formed large inclusions (Figs. 3 *d*, 4 *a*, and 5 *c*), which electron microscopy revealed to be composed of IFs (Fig. 5 *d*). The level of vimentin accumulation can be very high, and the cell can become essentially packed full of vimentin filaments (Fig. 5 *c*). Exogenous desmin, in contrast, behaved very much like endogenous desmin, i.e., it was associated primarily with the sarcolemma and formed a fine cytoplasmic meshwork (Fig. 3 *a*). At moderate and

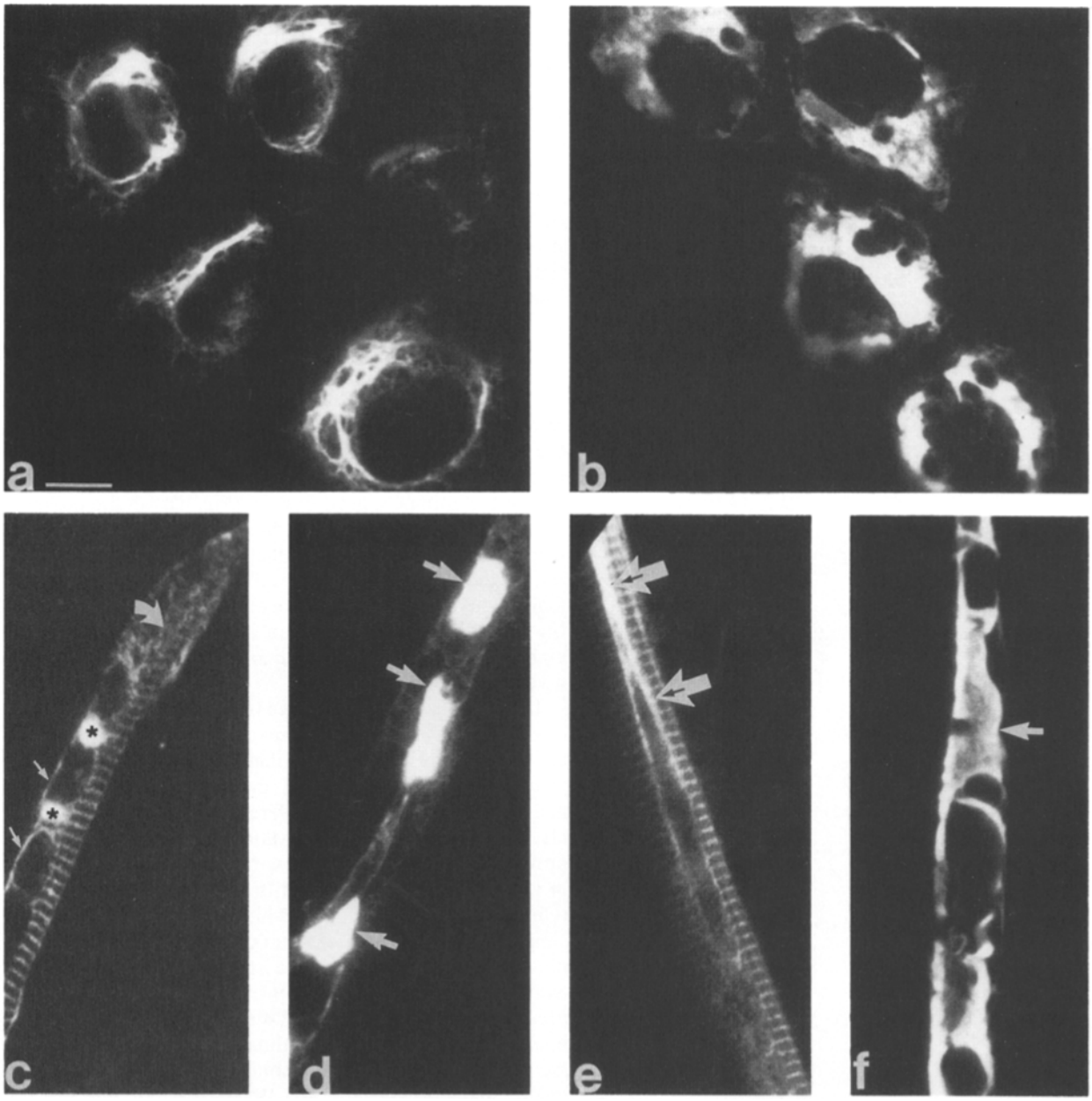

Figure 7. Chimeras in embryonic epithelia and myocytes. Fertilized eggs were injected with linear pCskAct.DVV or pCskAct.VDD DNA, fixed at stage 38/40 and stained in whole-mount with 9E10 and fluorescein-conjugated secondary antibody. Embryonic epithelial cells accumulating DVV formed discrete IF networks (*a*) whereas VDD (*b*) formed large densely staining inclusion bodies. At moderate levels in myotomal myocytes, DVV formed fine filament meshworks (*curved arrow* in *c*), aggregates ("*") and associated with the sarcolemma (*arrows*) and Z-lines (*c*). At higher levels of accumulation the distribution of DVV was much the same though aggregates were somewhat more prominent (*arrows* in *d*). At moderate levels of accumulation VDD formed longitudinal filaments (*arrow* in *e*) and associated with the Z-lines. At high accumulation levels VDD formed large inclusion bodies (*arrow* in *f*). Bar in *a* marks 10 μm for *a–f*.

high levels of accumulation, cells also contained discrete aggregates (Fig. 3 *b*). These aggregates were not unlike those formed by endogenous desmin in some late stage tadpole myocytes (see Cary and Klymkowsky, 1994). Immunoelectron microscopy reveals that these aggregates are composed largely of desmin in a non-filamentous form (Fig. 5, *a* and *b*).

The NH_2-terminal Head Domains Determine Vimentin/Desmin Behavior

The amino acid sequence of vimentin and desmin differ most dramatically in the NH_2-terminal head region (Fig. 6). To determine whether these differences in sequence produced the differences in vimentin and desmin organization seen in both epithelial and muscle cells, we took the head of one protein and fused it to the body of the other. These are seam-less fusions with no added to altered amino acids; moreover the "cross-over" point was selected to be in a region of high homology (Figs. 1 *c* and 6). The behaviors of the two chimeric proteins were examined in epithelia and muscle. In embryonic epithelial cells, DVV formed a network of dis-

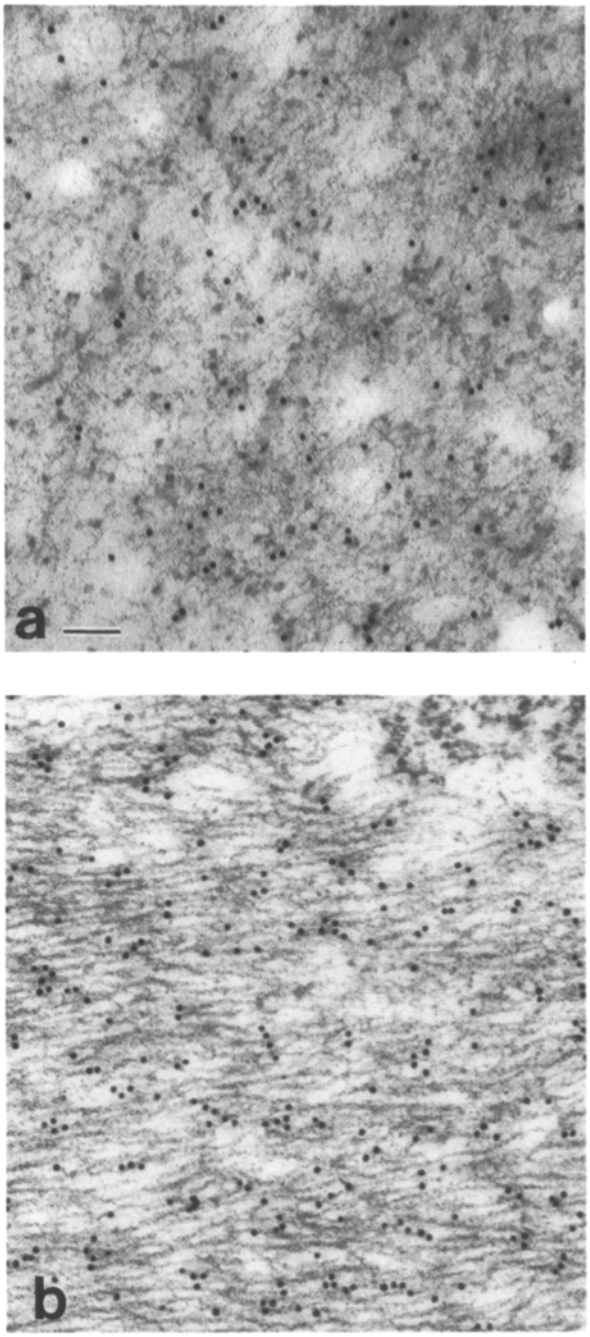

Figure 8. Ultrastructural analysis of myocytes expressing chimeric proteins. Electron microscopic analysis of myocytes expressing moderate to high levels of DVV reveals the presence of 9E10-reactive aggregates composed of granular, non-filamentous material (*a*). VDD, on the other hand, forms inclusion bodies composed of densely packed IFs (*b*). Bar in *a* marks 100 nm in *a* and *b*.

crete filaments (Fig. 7, *a*), while VDD formed large aggregates that appeared, at the light microscopic level, identical to the aggregates of exogenous vimentin seen in myocytes (compare Figs. 3 *d*-7 *b*). In muscle, the chimeric proteins behaved like the protein from which their head domains were derived. DVV expressing myocytes formed reticular filament meshworks and discrete, punctate aggregates similar to those observed for endogenous and exogenous desmin (Fig. 7, *c* and *d*). VDD, on the other hand, formed longitudinal IF arrays and massive inclusions like those seen in vimentin-expressing myocytes (Figs. 4, *b* and *c* and 7, *e* and *f*). The similarity of DVV to endogenous and exogenous desmin and the similarity of VDD to exogenous vimentin extended to the ultrastructural level: DVV formed aggregates composed of finer structures, perhaps protofilaments (Fig. 8 *a*) whereas VDD formed massive bundles of 10-nm filaments (Fig. 8 *b*). These results point to the head domains as the primary determinant of vimentin/desmin behavior in muscle. In addition, it is clear from the differential organization of VDD and DVV in embryonic epithelial cells (Fig. 7, *a* and *b*), that the two head domains are not structurally equivalent.

The behavior of the chimeric proteins is also significant in terms of ruling out possible effects of the tagging epitope on the differential behavior of the exogenous vimentin and desmin proteins. In VDD, the tail-tagging region is identical to that in the epitope tagged desmin (Fig. 1), yet the polypeptide behaves like vimentin. Similarly, in DVV the tail-tagging region is identical to that of epitope-tagged vimentin, yet the polypeptide behaves like desmin. Moreover, both tagged vimentin and desmin are capable of de novo IF assembly in both IF-free mammalian cells (Fig. 2, *a* and *b*) and embryonic epithelia (Fig. 2, *c* and *d*), which contain only a keratin-type IF system.

The conclusion of our studies is that vimentin and desmin clearly differ in the types of structures they form in both muscle and non-muscle cells and that these differences are due primarily to the sequence of the NH_2-terminal head domain. Exactly what these differences are, however, is not clear. Considering the observation that the neurofilament protein NFL, which can be assembled into 10-nm filaments in vitro fails to form extended IF networks by itself in vivo (Ching and Liem, 1993; Lee et al., 1993), it is clear that the simple fact that purified desmin can be assembled into 10-nm filaments in vitro does not imply that it will be able to assemble into IFs under various in vivo conditions. We have previously found evidence that the head domain of vimentin interacts with factors in the animal hemisphere of the *Xenopus* oocyte that influence the ability of vimentin to form extended filaments (Dent et al., 1992). It is possible that the head domains of vimentin and desmin differ in their ability to interact with cytoplasmic factors present in embryonic epithelia and muscle and that these interactions modify the type of IF networks they form. It is also known that phosphorylation can play a role in determining the assembly properties of IF proteins, including desmin (see Geisler and Weber, 1988). the majority of vimentin's and desmin's phosphorylated residues occur in the head domain (Evans, 1988; Geisler and Weber, 1988; Geisler et al., 1989; Christian et al., 1990; Dent et al., 1992) and it is possible that differences in the head domains of vimentin and desmin lead to differences in the pattern of phyosphorylation. In the myocyte, phosphorylation could affect desmin's ability to form extended filaments, alternatively the modified desmin might require a host cell factor to assemble filaments. Once the level of desmin accumulates above some threshold, the factor required for IF formation may become limiting, leading to the formation of desmin aggregates rather than filaments.

Defects in Muscle Structure Due to Overexpression of IF Proteins

The defects in muscle structure induced by the overexpression of desmin and the ectopic expression of vimentin are not unexpected given the work of Dunia et al. (1990). They found that ectopically expressed hamster desmin in the mouse lens induced defects in membrane and cellular structure (see also Capetanaki et al., 1989). We see distinct morphological abnormalities associated with the overexpression of desmin and with the ectopic expression of vimentin. Desmin overexpression often results in a wrinkling of the cell. The wrinkled appearance may be indicative of membrane defects resulting from the overaccumulation of desmin in the subsarcolemmal region. This defect may also be a by-product of aberrant mechanical coupling of the myofibrils to the sarcolemma. In any case, the defect is distinct from that observed in cells accumulating large amounts of exogenous vimentin. These cells typically display a swelling that corresponds to the morphology and location of the vimentin inclusion. Commonly, vimentin inclusion bodies localized to the central region of the cell appear to produce spindle-shaped myocytes, whereas inclusions localized to one end of the cell produce "club"-shaped myocytes. It seems likely that the presence of large vimentin inclusions physically distorts the cell. High levels of vimentin or desmin accumulation can lead to the detachment of the myocyte from the intersomite junction or myoseptum.

A Prediction Concerning Myopathies

Although the levels of exogenous proteins generated in these studies are not likely to reflect common pathological conditions, one potentially important observation does arise from this work. A number of myopathies and cardiomyopathies have been characterized by the presence of IF inclusions (Sakakibara et al., 1970; Shafiq et al., 1974; Edstrom et al., 1980; Porte et al., 1980; Fidzianska et al., 1983; Pellissier et al., 1989; Sarnat, 1990, 1991, 1992; Halbig et al., 1991; Telerman et al., 1991; D'Amati et al., 1992; for review see Goebel and Bornenmann, 1993). Ultrastructural analysis of these inclusions reveals a range of appearances from granular to filamentous. Unfortunately, reports that examine both the ultrastructure and the composition of such inclusions (i.e., whether they contain desmin, vimentin, or both proteins) are scarce. Although vimentin is not normally present in adult skeletal or cardiac muscle, vimentin has been found in a number of myopathies (Sarnat, 1991, 1992). Based on the differential behavior of vimentin and desmin in the *Xenopus* myotome, we predict that myopathies characterized by the accumulation of intact IFs will be found to correlate with the expression of vimentin. On the other hand, a number of congenital myopathies have been sown to involve the aberrant accumulation of desmin (Edstrom et al., 1980; Fidzianska et al., 1983; Pellissier et al., 1989; Telerman et al., 1991). We would predict that an accumulation of desmin, in the absence of vimentin, would result in nonfilamentous inclusions, similar to those seen when exogenous desmin is synthesized in *Xenopus* muscle (Fig. 5, *a* and *b*). Given the distinctive difference in the cellular morphologies of myocytes overexpressing vimentin or desmin (Fig. 3, *f* and *g*) it seems likely that the overexpression of these proteins will lead to distinct defects in the muscle cell.

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References

Bachant, J. B. 1993. Assembly and dynamics of keratin intermediate filaments in Xenopus laevis oocytes. Ph.D. thesis. University of Colorado, Boulder CO.

Bennett, G. S., S. A. Fellini, Y. Toyama, and H. Holtzer. 1979. Redistribution of intermediate filament subunits during skeletal myogenesis and maturation in vitro. *J. Cell Biol.* 82:577–584.

Blessing, M., U. Ruther, and W. W. Franke. 1993. Ectopic synthesis of epidermal cytokeratins in pancreatic islet cells of transgenic mice interferes with cytoskeletal order and insulin production. *J. Cell Biol.* 120:743–755.

Brady, A. J. 1984. Passive stiffness of rat cardiac myocytes. *J. Biomech. Eng.* 106:25–30.

Capetanaki, Y., S. Smith, and J. P. Heath. 1989. Over-expression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. *J. Cell Biol.* 109:1653–1664.

Cary, R. B., and M. W. Klymkowsky. 1994. Desmin organization during the differentiation of the dorsal myotome in *Xenopus laevis*. *Differentiation.* 56:31–38.

Ching, G. Y., and R. K. Liem. 1993. Assembly of type IV neuronal intermediate filaments in non-neuronal cells in the absence of preexisting cytoplasmic intermediate filaments. *J. Cell Biol.* 122:1323–1335.

Christian, J. L., N. G. Edelstein, and R. T. Moon. 1990. Over-expression of wild-type and dominant negative mutant vimentin subunits in developing Xenopus embryos. *New Biol.* 2:700–711.

Cossette, L., and J. M. Vincent. 1991. Expression of a developmentally regulated cross-linking intermediate filament-associated protein (IFAPa-400) during the replacement of vimentin for desmin in muscle cell differentiation. *J. Cell Sci.* 98:251–260.

Côté, F., J.-P. Collard, and J.-P. Julien. 1993. Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell.* 73:35–46.

Craig, S. W., and J. V. Pardo. 1983. γ-actin, spectrin, and intermediate filament proteins colocalize with vinculin at costameres, myofibril-to-sarcolemma attachment sites. *Cell Motil.* 3:449–462.

Cross, G. S., C. Wilson, H. P. Erba, and H. R. Woodland. 1988. Cytoskeletal actin gene families of Xenopus borealis and Xenopus laevis. *J. Mol. Evol.* 27:17–28.

D'Amati, G., H. J. Kahn, J. Butany, and M. D. Silver. 1992. Altered distribution of desmin filaments in hypertrophic cardiomyopathy: an immunohistochemical study. *Mod. Pathol.* 5:165–168.

Dent, J. A., R. B. Cary, J. B. Bachant, A. Domingo, and M. W. Klymkowsky. 1992. Host cell factors controlling vimentin organization in the Xenopus oocyte. *J. Cell Biol.* 119:855–866.

Dent, J. A., A. G. Polson, and M. W. Klymkowsky. 1989. A whole-mount immunocytochemical analysis of the expression of the intermediate filament protein vimentin in Xenopus. *Development.* 105:61–74.

Dunia, I., F. Pieper, S. Manenti, A. van de Kemp, G. Devilliers, E. L. Benedetti, and H. Bloemendal. 1990. Plasma membrane-cytoskeleton damage in eye lenses of transgenic mice expressing desmin. *Eur. J. Cell. Biol.* 53:59–74.

Edstrom, L., L. Thornell, and A. Eriksson. 1980. A new type of hereditary distal myopathy with characteristic sarcoplasmic bodies and intermediate (skeleton) filaments. *J. Neurol. Sci.* 47:171–190.

Evans, R. M. 1988. The intermediate-filament proteins vimentin and desmin are phosphorylated in specific domains. *Eur. J. Cell. Biol.* 46:152–160.

Eyer, J. A., and A. Peterson 1994. Neurofilament deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-β-galactosidase fusion protein. *Neuron.* 12:389–405.

Fidzianska, A., H. H. Goebel, M. Osborn, H. G. Lenard, G. Osse, and U. Langenbeck. 1983. Mallory body-like inclusions in a hereditary congenital neu-

romuscular disease. *Muscle & Nerve*. 6:195–200.
Gard, D. L., and E. Lazarides. 1980. The synthesis and distribution of desmin and vimentin during myogenesis in vitro. *Cell*. 19:263–275.
Geisler, N., N. Hatzfeld, and K. Weber. 1989. Phosphorylation of vimentin by protein kinase A and C is restricted to the head domain: identification of the phosphoserine sites and their influence on filament formation. *Eur. J. Biochem*. 183:441–447.
Geisler, N., and K. Weber. 1988. Phosphorylation of desmin in vitro inhibits formation of intermediate filaments; identification of three kinase A sites in the aminoterminal head domain. *EMBO (Euro. Mol. Biol. Organ.) J.* 7:15–20.
Gill, S. R., P. C. Wong, M. J. Monteiro, and D. W. Cleveland. 1990. Assembly properties of dominant and recessive mutations in the small mouse neurofilament (NF-L) subunit. *J. Cell Biol*. 111:2005–2019.
Goebel, H., and H. A. Bornenmann. 1993. Desmin pathology in neuromuscular disease. *Virchows Arch. B. Cell. Pathol*. 64:127–135.
Granger, B. L., and E. Lazarides. 1979. Desmin and vimentin coexists at the periphery of the myofibril Z disc. *Cell*. 18:1053–1063.
Halbig, L., H. H. Goebel, H. C. Hopf, and R. Moll. 1991. Spheroid-cytoplasmic complexes in a congenital myopathy. *Rev. Neurol. (Paris)*. 147:300–307.
Hemsley, A., N. Arnheim, M. D. Toney, G. Cortopassi, and D. J. Galas. 1989. A simple method for site-directed mutagenesis using the polymerase chain reaction. *Nucleic Acids Res*. 17:6545–6551.
Herrmann, H., B. Fouquet, and W. W. Franke. 1989*a*. Expression of intermediate filament proteins during development of Xenopus laevis. I. cDNA clones encoding different forms of vimentin. *Development*. 105:279–298.
Herrmann, H., B. Fouquet, and W. W. Franke. 1989*b*. Expression of intermediate filament proteins during development of Xenopus laevis. II. Identification and molecular characterization of desmin. *Development*. 105: 299–307.
Herrmann, H., A. Eckelt, M. Brettel, C. Grund, and W. W. Franke. 1993. Temperature-sensitive intermediate filament assembly. Alternative structures of Xenopus laevis vimentin in vitro and in vivo. *J. Mol. Biol*. 234:99–113.
Hoffman, W., J. K. Franz, and W. W. Franke. 1985. Amino acid sequence microheterogeneities of basic (type II) cytokeratins of Xenopus laevis epidermis and evolutionary conservativity of helical and non-helical domains. *J. Mol. Biol*. 184:713–724.
Hoffman, P. N., E. H. Koo, N. A. Muma, J. W. Griffin, and D. L. Price. 1988. Role of neurofilaments in the control of axonal calibre in myelinated nerve fibers. *In* Intrinsic Determinants of Neuronal Form. R. Liss, New York, NY. 389–402.
Holtzer, H., G. S. Bennett, S. J. Tapscott, J. M. Croop, and Y. Toyama. 1982. Intermediate-sized filaments: changes in synthesis and distribution in cells of the myogenic and neurogenic lineages. *Cold Spring Harb. Symp. Quant. Biol*. 42:317–329.
Klymkowsky, M. W. 1981. Intermediate filaments in 3T3 cells collapse after intracellular injection of a monoclonal anti-intermediate filament antibody. *Nature (Lond.)*. 291:249–251.
Klymkowsky, M. W., and J. Hanken. 1991. Whole-mount staining of Xenopus and other vertebrates. *In* Xenopus laevis: Practical Uses in Cell and Molecular Biology. B. K. Kay and H. B. Peng, editors. Academic Press, Ltd, San Diego. 419–441.
Klymkowsky, M. W., D. R. Shook, and L. A. Maynell. 1992. Evidence that the deep keratin filament system of the Xenopus embryo acts to ensure normal gastrulation. *Proc. Natl. Acad. Sci. USA*. 89:8726–8740.
Lazarides, E. 1980. Intermediate filaments as mechanical integrators of cellular space. *Nature (Lond.)*. 283:249–256.
Lee, M. K., Z. Xu, P. C. Wong, and D. W. Cleveland. 1993. Neurofilaments are obligate heteropolymers in vivo. *J. Cell Biol*. 122:1337–1350.
Ohara, O., Y. Gahara, T. Miyake, H. Teraoka, and T. Kitamura. 1993. Neurofilament deficiency in Quail caused by nonsense mutation in neurofilament-L gene. *J. Cell Biol*. 121:387–395.
Pellissier, J. F., J. Pouget, C. Charpin, and D. Figarella. 1989. Myopathy associated with desmin type intermediate filaments. An immunoelectron microscopic study. *J. Neurol. Sci*. 89:49–61.
Porte, A., M. Stoeckel, A. Sacrez, and A. Batzenschlager. 1980. Unusual familial cardiomyopathy with storage of intermediate filaments in the cardiac muscular cells. *Virchows Arch*. 386:43–58.
Quinlan, R. A., and W. W. Franke. 1982. Heteropolymeric filaments of vimentin and desmin in vascular smooth muscle tissue and cultured baby hamster kidney cells demonstrated by chemical crosslinking. *Proc. Natl. Acad. Sci. USA*. 79:3452–3456.
Sakakibara, S., M. Sekiguchi, S. Konno, and M. Kusumoto. 1970. Idiopathic postpartum cardiomyopathy: report of a case with special reference to its ultrastructural changes in the myocardium as studied by endomyocardial biopsy. *Am. Heart J*. 80:385–395.
Sarnat, H. B. 1990. Myotubular myopathy: arrest of morphogenesis of myofibers associated with persistence of fetal vimentin and desmin. Four cases compared with fetal and neonatal muscle. *Can. J. Neurol. Sci*. 17:109–123.
Sarnat, H. B. 1991. Vimentin/desmin immunoreactivity of myofibers in developmental myopathies. *Acta Paediatr. Jpn*. 33:238–246.
Sarnat, H. B. 1992. Vemintin and desmin in maturing skeletal muscle and developmental myopathies. *Neurology*. 42:1616–1624.
Sarria, A. J., S. K. Nordeen, and R. M. Evans. 1990. Regulated expression of vimentin cDNA in cells in the presence and absence of a preexisting vimentin filament network. *J. Cell Biol*. 111:553–565.
Sarria, A. J., S. R. Panini, and R. M. Evans. 1992. A functional role for vimentin intermediate filaments in the metabolism of lipoprotein-derived cholesterol in human SW-13 cells. *J. Biol. Chem*. 267:19455–19463.
Sejersen, T., and U. Lendahl. 1993. Transient expression of the intermediate filament nestin during skeletal muscle development. *J. Cell Sci*. 106: 1291–1300.
Shafiq, S. A., M. A. Sande, R. R. Carruthers, T. Killip, and A. T. Milhorat. 1974. Skeletal muscle in idiopathic cardiomyopathy. *J. Neurol. Sci*. 15: 303–320.
Smith, W. C., and R. M. Harland. 1991. Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing center. *Cell*. 67:753–765.
Steinert, P. M., W. W. Idler, F. Cabral, M. M. Gottesman, and R. D. Goldman. 1981. In vitro assembly of homopolymer and copolymer filaments from intermediate filament subunits of muscle and fibroblastic cells. *Proc. Natl. Acad. Sci. USA*. 78:3692–3696.
Takebe, Y., M. Seike, J. Fujisawa, P. Hoy, K. Yokota, K. Arai, M. Yoshida, and N. Arai. 1988. SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol*. 8:466–472.
Telerman, T. N., G. Bauherz, and S. Noel. 1991. Auriculo-ventricular block and distal myopathy with rimmed vacuoles and desmin storage. *Clin. Neuropathol*. 10:61–64.
Tidball, J. G. 1992. Desmin at myotendinous junctions. *Exp. Cell Res*. 199:206–212.
Tokuyasu, K. T., P. A. Maher, and S. J. Singer. 1985. Distributions of vimentin and desmin in developing chick myotubes in vivo. II. Immunoelectron microscopic study. *J. Cell Biol*. 100:1157–1166.
Traub, P. 1985. Intermediate filaments: a review. Springer-Verlag, Berlin.
van de Klundert, F. A. J. M., J. M. H. Raats, and H. Bloemendal. 1993. Intermediate filaments: regulation of gene expression and assembly. *Eur. J. Biochem*. 214:351–366.
Vize, P. D., D. A. Melton, A. Hemmati-Brivanlou, and R. M. Harland. 1991. Assays for gene function in developing Xenopus embryos. *In* Xenopus laevis: Practical Uses in Cell and Molecular Biology. B. K. Kay and H. B. Peng, editors. Academic Press, Ltd., San Diego, CA. 367–387.
Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA*. 76:615–619.
Wong, P. C., and D. W. Cleveland. 1990. Characterization of dominant and recessive assembly-defective mutations in mouse neurofilament NF-M. *J. Cell Biol*. 111:1987–2003.
Xu, Z., L. C. Cork, J. W. Griffin, and D. W. Cleveland. 1993. Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell*. 73: 23–33.