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Phosphorylation of desmin *in vitro* inhibits formation of intermediate filaments; identification of three kinase A sites in the aminoterminal head domain

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The *in vitro* phosphorylation of chicken desmin by the catalytic subunit of cAMP-dependent protein kinase was analysed. Phosphorylated desmin loses the ability to form intermediate filaments (IFs). Fragmentation at the sole cysteine and mild chymotryptic treatment show a differential phosphorylation of the three structural domains. Only the amino-terminal head domain is the target of the kinase. Peptide analysis shows that serine 29 is fully phosphorylated, while serine 35 and 50 are phosphorylated at least at 22 and 50% respectively. All three sites show the sequence arginine-X-serine with X being a small residue. These results strengthen the view that the non-helical head domain has a strong influence on filament integrity most likely via a direct influence of some of its arginine residues. Taken together with previous results (Inagaki *et al.*, 1987) on the phosphorylation of vimentin by kinase A, a new view on IFs emerges. Phosphorylation could allow for regulatory processes in assembly and turnover.

Key words: cytoskeleton/desmin/intermediate filaments/phosphorylation/protein kinase A/serine-phosphate

Introduction

Little is known about the intracellular turnover of intermediate filaments (IFs) (Traub, 1985). These structures are rather insoluble *in vitro* under physiological salt and pH conditions and only a small pool of unpolymerized material is found (Soellner *et al.*, 1985). All current models of IF structure (Geisler *et al.*, 1985; Ip *et al.*, 1985; Steinert *et al.*, 1985; Fraser *et al.*, 1986) assume that the filament wall is primarily based on the interaction patterns of double-stranded coiled-coils (Geisler and Weber, 1982) provided by the central α-helical rod domains of the proteins. Defined proteolytic derivatives indicate, however, that the terminal non-α-helical domains have a direct effect on filament stability and polymerization (Geisler *et al.*, 1982; Nelson and Traub, 1983; Traub and Vorgias, 1983). Specific removal of the amino-terminal head domain of chicken desmin by thrombin provides a protein unable to polymerize on its own. In contrast, half of the carboxy-terminal tail domain of desmin can be removed by lysine-protease without an effect of filament-forming ability (Kaufmann *et al.*, 1985). Thus at least for desmin and vimentin particular emphasis has been put on the arginine-rich head domain as a region influencing filament stability. As the head domain is the preferred target of the calpains (Fischer *et al.*, 1986), proteolytic destruction of IFs *in vivo* can be explained (Traub, 1985).

There are many *in vivo* and *in vitro* phosphorylation studies on vimentin and desmin (Cabral and Gottesman, 1979; Gard *et al.*, 1979; O'Connor *et al.*, 1981; Evans and Fink, 1982; Gard and Lazarides, 1982; Celis *et al.*, 1983; Evans, 1984) but a direct relation between this modification and filament stability was not drawn for many years. Although isoproteronol, for instance, is known to stimulate the phosphorylation of desmin and vimentin in cultured chicken myotubes (O'Connor *et al.*, 1981; Gard and Lazarides, 1982), the possible implications of the phosphorylation were not followed up. One reason for a lack of further studies on the action of protein kinase A on IFs may be connected with the protein sequences. Neither desmin nor vimentin reveal the hallmark of the good kinase A substrate site (Geisler and Weber, 1982; Quax *et al.*, 1983). This is thought be a cluster of two or three basic residues, usually arginine, separated by one residue from a serine or threonine (reviewed by Edelman *et al.*, 1987). Recently, however, Inagaki *et al.* (1987) found that vimentin phosphorylated *in vitro* by protein kinase A becomes polymerization incompetent. Stimulated by their report we decided to study desmin phosphorylation *in vitro* by kinase A. Desmin rather than vimentin was used for two reasons. Lazarides and co-workers showed several years ago that desmin phosphorylated *in vitro* by kinase A shares several tryptic phosphopeptides with *in vivo* phosphorylated desmin (O'Connor *et al.*, 1981; Gard and Lazarides, 1982). In addition, the methods employed during the sequence analysis of desmin seemed suitable to assign rapidly the phosphate positions along the three-domain structure of IF proteins (Geisler and Weber, 1981,1982; Geisler *et al.*, 1982). Here we show that desmin phosphorylated by kinase A loses its ability to form normal IFs. Phosphorylation occurs exclusively in the head domain and the three identified serine-phosphate sites lie in close proximity to an arginine. These results connect the previous view of desmin filament structure with the notion that phosphorylation could influence intracellular depolymerization and turnover of IFs.

Results

Phosphorylation sites locate to the head domain

Chicken desmin was treated with kinase A using conditions similar to those reported by Inagaki *et al.* (1987) for the phosphorylation of vimentin by the same enzyme. Under these conditions (see Materials and methods) ~2.3 mol of phosphates were incorporated per mol of desmin (mol. wt 53 000). To locate the phosphate sites, standard procedures developed during the sequence analysis of desmin were applied. Cleavage with 2-nitro-5-thiocyanobenzoic acid at the sole cysteine provided two fragments in addition to unreacted protein (Geisler and Weber, 1981). Gel electrophoresis and

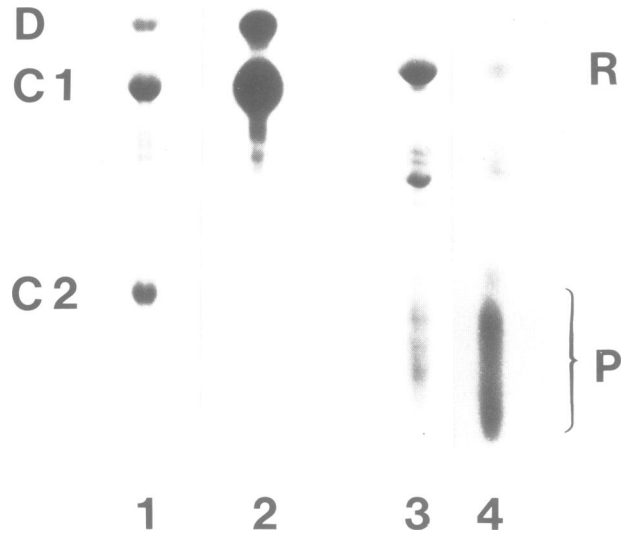

Fig. 1. Analysis of ^{32}P label after phosphorylation of chicken desmin by the catalytic subunit of kinase A. Phosphorylated desmin (D) cleaved at the single cysteine (**slot 1**) gives two fragments C1 and C2. Autoradiography (**slot**) shows that the label is restricted to the amino-terminal C1 fragment. Direct counting showed 18 000 c.p.m. for C1 and 45 c.p.m. for C2. Phosphorylated desmin mildly treated with chymotrypsin (**slot 3**) to excise the rod domain (R) shows label nearly exclusively (**slot 4**) in the smaller peptides (P) resulting from the head domain (see Figures 2 and 3). Direct counting after destaining gave 3400 c.p.m. for the peptide region and 200 c.p.m. for the rod (see text). Gels 1 and 3 were run on different occasions.

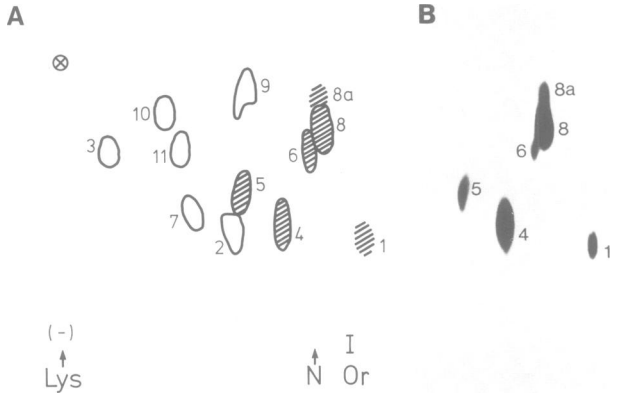

Fig. 2. Two-dimensional fingerprint (**A**) and corresponding autoradiogram (**B**) of the peptides released from phosphorylated desmin by mild treatment with chymotrypsin. The origin (Or) is marked. Electrophoresis at pH 6.5 was followed by descending chromatography (n-butanol, acetic acid, pyridine and water). The larger part of the acidic side (right) is not shown as no peptides were present. Lys and N mark the position of free lysine and the neutral amino acid valine used as electrophoretic markers. The circled X indicates the position of the xylene xyanol FF used as marker during chromatography. Peptides were detected with fluorescamin. Hatched spots in A correspond to radioactive spots in B. Peptides 1 and 8a did not react with fluorescamin as they lack a free amino terminus as well as lysine (Figure 3). Numbering of peptides is according to the desmin sequence (see Figure 3). The total radioactivity (c.p.m.) was 416 000 (peptide 1), 1 240 000 (peptide 4), 500 000 (peptide 5), 260 000 (peptide 6), 1 400 000 (peptide 8) and 360 000 (peptide 8a).

autoradiography showed that virtually all ^{32}P-label resides in the amino-terminal fragment C1 (residues 1–323) while the second fragment C2 (residues 324–463) was practically unlabelled (Figure 1, slots 1 and 2). A mild chymotryptic digest of desmin provided the rod domain (residues 70–415), the tail domain (residues 416–463) and the various peptides accounting for the head domain (residues 1–69) (Geisler *et al.*, 1982). Figure 1 (slots 3 and 4) documents the heavy labelling of the peptides from the head region. About 6% of the label is found in the rod while 94% correspond to small peptides. As the gel was processed through dye staining and destaining, a value of 94% is an underestimate, since some peptide material is lost upon destaining. The combined results locate the major phosphorylation sites to the head domain. Phospho amino acid analysis documented exclusive serine phosphorylation.

Identification of the serine phosphate sites in the head domain

For the location studies we followed again the previous sequence approach. The peptides comprising the head domain in a mild chymotryptic digest were extracted with pH 6.5 electrophoresis buffer and separated by a two-dimensional fingerprint. pH 6.5 was used to obtain the information provided by the Offord plot. Figure 2 shows the fingerprint and the corresponding autoradiograph. The properties established on the peptides are summarized in Figure 3. All 12 peptides arise from the known sequence of the head domain as defined previously (Geisler *et al.*, 1982). This conclusion based on amino acid composition was extended by gas-phase sequencing of the longer peptides. There are two chymotryptic peptides with a blocked amino-terminal end. Peptide 1 was characterized by a secondary tryptic digest. Cleaving at the sole arginine, the carboxy-terminal sequence following this residue was identified. Peptide 1 start at the acetylated residue 1 (see Figure 3). Peptide 8a starting at residue 45 arises by cyclization of the amino-terminal glutamine to a pyrrolidone-carboxyl residue. Yield values given in Figure 3 were normalized on the molar yield of the tetrapeptide number 3 (Figure 3).

Although there are six labelled chymotryptic peptides (Figure 2), these arise from four distinct phosphorylation sites (Figure 3) Three sites were readily identified. Peptides 4 and 5 both covered desmin residues 18–44. Using the Offord plot, peptide 4 contained two serine-phosphates, while peptide 5 had only one phosphate. In this analysis, which was controlled by the mobilities of the phosphorylated and unphosphorylated model peptide LRRASLG (kemptide), a phosphate group contributes one negative charge to the pH 6.5 net charge. Already during gas-phase sequencing, the 12th residue of both peptide 4 and 5 was identified as serine-phosphate using the analysis described by Meyer *et al.* (1986) as outlined in Materials and methods (procedure a). Thus serine 29 of desmin was identified in the phosphorylated form. The second serine-phosphate of peptide 4 seemed to involve serine 35 of desmin and this was verified by sequencing of the two radioactive tryptic peptides of peptide 4 (procedure b). Peptide 6 spanned residues 18–30 and had a single phosphorylation site corresponding to serine 29 of desmin (procedure a). The third distinct phosphorylation site of desmin was located in peptide 8. It was identified in the sixth position of the peptide (procedure a) and involves desmin residue 50. Since the corresponding peptide 8a was obtained in low yield and was blocked at the N terminus (see above), we did not follow the phosphate site up in detail. We tentatively assume that it involves serine 50 as shown

Peptides	Yield %	Phospho-serines	Position	
1 (res. 1 to 13)				
blSQSYSSSQRVSSY	15	1	(1 to 7)	
1T VSSY				
2 (res. 5 to 13)				
SSSQRVSSY	77	-		
3 (res. 14 to 17)				
RRTF	100	-		
4 (res. 18 to 44)				
GGGTSPVFPRA**S**FGSRG**S**GSSVTSRVY	22	2	29,35	(a)
4T1 A**S**FGSR		1	29	(b,c)
4T2 G**S**GSSVTSR		1	35	(b,c)
5 (res. 18 to 44)				
GGGTSPVFPRA**S**FGSRGSGSSVTSRVY	18	1	29	(a)
6 (res. 18 to 30)				
GGGTSPVFPRA**S**F	9	1	29	(a)
7 (res. 31 to 44)				
GSRGSGSSVTSRVY	18	-		
8 (res. 45 to 58)				
QVSRT**S**AVPTLSTF	50	1	50	(a)
8a (res. 45 to 58)				
blQVSRTSAVPTLSTF	13	1	(50?)	
9 (res. 45 to 58)				
QVSRTSAVPTLSTF	10	-		
10 (res. 59 to 66)				
RTTRVTPL	78	-		
11 (res. 67 to 69)				
RTY	98	-		

Fig. 3. Location of phosphoserines in the head domain of desmin after phosphorylation by kinase A. Chymotryptic peptides 1–11 from Figure 2 were characterized and placed along the desmin sequence (Geisler and Weber, 1982). Residue numbers are given in parenthesis. A blocked amino terminus is indicated by bl. This is an acetyl group in peptide 1 and a cyclized glutamine in 8a. Sequences determined are underlined. Peptides labelled T are secondary tryptic peptides. Yields are given in % relative to the yield of the tetrapeptide 3 which was taken as 100%. The number of phosphoserines in the chymotryptic peptides is based on the sequence and the electrophoretic mobility at pH 6.5 (Figure 2) using the Offord plot. The position of the phosphoserines indicated by bold letter type was deduced as in Materials and methods. Identification during sequencing of the chymotryptic peptides involving a serine provided exclusively as the DTT adduct of PTH-serine is procedure a. The same approach used on tryptic peptides is procedure b. Identification of free phosphate during manual Edman degradation uses procedure c. Note the identification of three phosphorylation sites involving serines 29, 35 and 50. A fourth phosphorylated serine, not yet characterized, lies in the region of residues 1–7 provided by peptide 1 (see Results).

for peptide 8. A fourth but minor phosphorylation site present in residues 1–13 was indicated by peptide 1, which is blocked by the acetyl group (see above). As this region contains seven serine residues and has a blocked end group, a detailed analysis was postponed. As a secondary tryptic digest of peptide 1 provided residues 10–13 (peptide 1T in Figure 3) without serine-phosphate, we tentatively assume that the fourth but minor site locates to desmin residues 1–7. We checked in additional electrophoretic patterns whether residues 1–4 as a separate chymotryptic peptide could carry a phosphate overlooked in our standard fingerprint. Using much shorter eletrophoretic separation times no labelled peptides moving faster toward the cathode than peptide 1 were detected.

The combined results showed that serine 29 is the major phosphorylation site of desmin. This serine residue seems stoichiometrically phosphorylated. It was only present in labelled chymotryptic peptides for which a serine-phosphate corresponding to serine 29 could be documented (peptides 4, 5 and 6). Serines 35 and 50 are additional but substoichiometric phosphorylation sites since for each site corresponding unlabelled peptides were isolated (Figure 3). Under our experimental conditions we assume that serine 50 is modified to at least 50%, while serine 35 was modified by at least 22%. The low-level phosphorylation site present in residues 1–7 was not further analysed. The serine-phosphates established at desmin positions 29, 35 and 50 reveal a similar linear sequence environment. This is characterized by an arginine separated by one residue from the phosphorylated serine (RXS). X is in all cases a small residue like alanine (site 29), glycine (site 35) or threonine (site 50).

Impaired filament formation of phosphorylated desmin

Polymerization competence of phosphorylated desmin was analysed parallel to normal desmin using the standard filament formation which results when desmin is dialysed from 8 M urea into filament buffer. Electron microscopy performed after negative staining revealed normal intermediate filaments (Figure 4B) for unmodified desmin serving as control. Phosphorylated desmin yielded abberant structures and no filaments. Instead, aggregates of protofilaments were visualized. These occurred often in unordered heaps (Figure 4A). When such dialysates were centrifuged in the Air-fuge (100 000 *g* for 10 min) nearly all unmodified desmin was pelleted, while phosphorylated desmin was harvested to only 50%. This shows that the phosphorylated desmin can aggregate but does so in a disordered fashion.

Discussion

We have shown that desmin is a good substrate for protein kinase A. The phosphorylated derivate loses its ability to form normal IFs. Our results confirm the report of Inagaki *et al.* (1987) for vimentin phosphorylation by kinase A, and in addition provide the first identification of the phosphorylation sites along the IF polypeptide. The identification of the phosphate sites has important implications for the understanding of the domain structure of IF proteins.

Phosphorylation of desmin by kinase A is restricted to the non-α-helical head domain and involves only serine residues. Peptide characterization identified four sites of which three could be characterized in detail. Serine 29 was stoichiometrically phosphorylated, while serines 35 and 50 were modified to at least 22 and 50% respectively. A fourth but minor phosphorylation site involving a serine in positions 1–7 was not further characterized. All three major phosphorylation sites share the sequence motif arginine-X-serine, with X being a small amino acid residue such as glycine, alanine or threonine. The importance of the sequences surrounding the desmin phosphorylation sites and the contribution of the secondary structure involved are currently not known. Interestingly, the chymotrypsin-resistant tail domain contains the sequence DQRGSEV at residues 427–433 (Geisler and Weber, 1981;1982). Conformational restrictions or the presence of two negative changes close to this RXS sequence may be responsible for a lack of phosphorylation at the serine. The two RSS sequences present in the head domain of vimentin (Fischer *et al.*, 1986; Quax *et al.*, 1983) are, however, likely candidates for the kinase A-catalysed

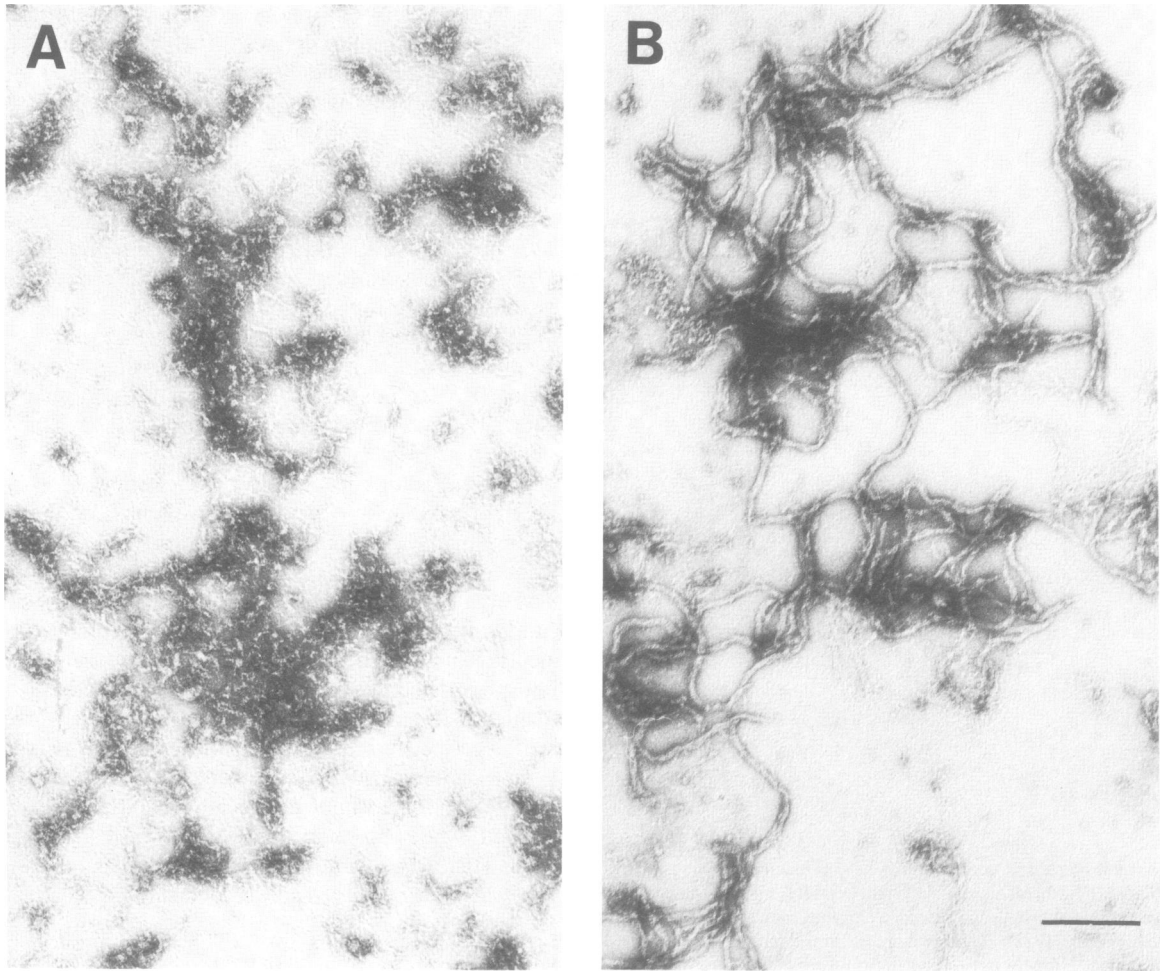

Fig. 4. Polymerization behavior of phosphorylated desmin (**A**) compared with unmodified desmin (**B**) serving as control. Proteins were dialysed at 0.2 mg/ml from 8 M urea into polymerization buffer (10 mM Tris−HCl, pH 7.5, 170 mM NaCl, 10 mM 2-mercaptoethanol, 0.2 mM $MgCl_2$) for 20 h at 4°C. After negative staining with uranyl acetate, micrographs were taken for both samples at the same magnification. Bar measures 0.2 μm. Note that phosphorylated desmin forms only aggregates of protofilaments (A) and not the typical IFs (B).

phosphorylation of vimentin observed by Inagaki *et al.* (1987).

The domain location of the major phosphorylation sites and their sequence environment agrees very well with certain aspects of IF filament structure developed by *in vitro* experiments on desmin (Geisler *et al.*, 1985; Kaufmann *et al.*, 1985). Although the filament wall seems to arise from an interaction pattern of the double-stranded coiled-coils provided by the rod domain, the non-α-helical terminal domains seem to provide a stabilizing factor, although they are thought to protrude from the wall. Use of defined proteolytic derivatives of desmin has put particular emphasis on the head domain and raised the speculation that the numerous arginine residues may have a direct effect on the polymerization process. Our results on the inhibition of desmin filament formation after phosphorylation by kinase A support this view. All three major phosphorylation sites occur very close to an arginine residue. Interestingly most IF proteins have head domains which are basic in nature. This feature arises from regions rich in arginine and nearly devoid of either lysine or acidic residues. Thus the introduction of the negatively charged phosphates may impair the contribution of the closely spaced arginines. Whether these are involved in setting the 22-nm half-unit length repeat between neighbouring IF protein units remains to be seen. In preliminary studies we found that desmin, just like vimentin (Inagaki *et al.*, 1987) can also be phosphorylated by protein kinase C. As this phosphorylation of desmin occurs exclusively in the amino-terminal fragment obtained by cleavage at cysteine, it most likely involves again the head domain.

The aggregates of protofilaments seen when desmin phosphorylation by kinase A is put under *in vitro* polymerization conditions, raise some interesting connections to previous ultrastructural studies. Many epithelial cell lines show breakdown of keratin filaments and the formation of aggregates of protofilamentous structures during mitosis (Franke *et al.*, 1982; Lane *et al.*, 1982). Such aggregates can also be sometimes induced under conditions leading to increased phosphorylation by kinase C (Toelle *et al.*, 1987). Although first experiments indicating increased keratin phosphorylation during mitosis (Celis *et al.*, 1983) have not been followed up, they and similar studies on vimentin (Evans and Fink, 1982; Evans, 1984), may be indicative of at least a partial IF disorganization due to phosphorylation. Indeed a transient change in the organization of vimentin filaments during mitosis has been reported in some cell lines studied with a particular monoclonal antibody (Franke *et al.*, 1984). However, not all phosphorylation sites of IF proteins are connected with impaired filament formation. The multiple phosphorylation sites present in the high mol. wt polypep-

tides M and H of neurofilaments do not interfere during *in vitro* co-assembly with component L (Julien and Mushynski, 1983; Geisler *et al.*, 1984; Georges *et al.*, 1986). These serine phosphate sites occur in the extended tail domains and mainly involve sequences of degenerate repetitive character unrelated to the desmin phosphorylation sites described here (Geisler *et al.*, 1987). *In vivo* phosphorylation of epidermal keratins is also well known, but phospho-peptides have been isolated for only one murine keratin (Steinert *et al.*, 1983). While the degree of phosphorylation and the precise position of the serine phosphates is not known, we note a potential RXS site in the peptide from the head domain. The two peptides from the tail domain have multiple serines and no RXS site.

Given the results of protein kinase A on desmin (this study) and vimentin (Inagaki *et al.*, 1987), we envisage that phosphorylation may influence many more interactions of cytoskeletal proteins than previously thought, since already certain arginine-X-serine sequences can act as good substrate sites for kinase A. For instance, the mitotic breakdown of the nuclear lamina seems to result from a transient phosphorylation of the lamins, which at least for lamins A and C is thought to yield the unassembled subunits (reviewed by Burke and Gerace, 1986). Although neither the protein kinase(s) involved nor the position of the phosphates is known, we found that the published lamin sequences (Fisher *et al.*, 1986; McKeon *et al.*, 1986; Krohne *et al.* 1987) point to some potential sites for kinase A. The rather short head domains of all lamins contain two consecutive basic residues separated by one amino acid from a serine or threonine. In addition, there are some sequences of the arginine-X-serine or threonine type in the unusually large tail domains. Recent *in vitro* studies emphasize that head and tails of IF proteins have additional functions. Heads of desmin and vimentin are implicated in plasma membrane interaction via a binding to ankyrin, while the tails show interaction with nuclear lamin B (Georgatos and Marchesi 1985; Georgatos and Blobel, 1987a,b; Georgatos *et al.*, 1987). Therefore corresponding binding studies with phosphorylated IF proteins should be explored in future studies.

Materials and methods

Phosphorylation

Chicken gizzard desmin purified in 8 M urea by standard procedures (Geisler and Weber, 1980) was dialysed at 0.5 mg/ml into 25 mM imidazole−HCl, pH 7, 30 mM NaCl. Prior to phosphorylation, $MgCl_2$, DTT and [^{32}P]ATP (100 Ci/mol) were added to 0.3, 0.5 and 0.1 mM respectively. The catalytic subunit of c-AMP protein kinase of beef heart, kindly provided by Dr H.D.Söling, was used at a final concentration of 0.009 mg/ml. After 4 h at 25°C the reaction was stopped by EDTA addition to 2.5 mM and lyophilization. Phosphorylated desmin was purified by gel filtration in 8 M urea, 10 mM Tris−HCl, pH 7.5, 1 mM 2-mercaptoethanol on a PD10 column (Pharmacia). Alternatively the protein was precipitated with 5 vol of ethanol from the reaction mixture, redissolved in 4 M guanidine−HCl and purified by HPLC on a C4 column. Phosphorylation was monitored by aliquots used for amino acid analysis and determination of phosphate incorporation by counting in Hydroluma plus (Baker). Radioactivity of desmin and its fragments (see below) separated by gel electrophoresis was first documented by autoradiography using Fuji-RX X-ray film. Subsequently the corresponding gel pieces were directly counted.

Domain assignment of phosphorylation sites

Cleavage of desmin at its sole cysteine with 2-nitro-5-thiocyanobenzoic acid was done as before (Geisler and Weber, 1981). A mild chymotryptic digest of desmin dialysed into 5 mM NH_4HCO_3 was prepared to provide the α-helical rod, the tail domain and the peptides spanning the head domain (Geisler *et al.*, 1982). A substrate to enzyme ratio of 400:1 was used for 10 min at 37°C. All procedures have been previously documented in our studies establishing the amino acid sequence of desmin. Fragments were analysed by gel electrophoresis and autoradiography.

Identification of the phosphorylation sites

Phosphorylated desmin (2.5 mg) was subjected to mild chymotryptic digestion (see above) and the reaction stopped by PMSF added to 1 mM and subsequent lyophilization. The soluble peptides of the head domain were extracted by pH 6.5 electrophoresis buffer (10% pyridine and 0.5% acetic acid in water) and subjected to the two-dimensional fingerprint system on Whatman 3 MM paper (Geisler *et al.*, 1982). Peptides were located by fluorescamine stain (0.0002% in acetone) followed by autoradiography. Eluted peptides were characterized by the following properties: quantitative amino acid analysis, determination of the molar amount of phosphate, phospho amino acid analysis and sequence analysis. Sequence analysis was on a gas-phase sequenator (model 470A; Applied Biosystems) equipped with an on-line PTH-amino acid analyser, using the 03 RPTH program of the manufacturer.

Several procedures were available to identify the serine phosphates along the known sequence of the desmin head domain (Geisler *et al.*, 1982; Geisler and Weber, 1982). During gas-phase sequencing, normal serine residues are identified as phenylthiohydantoin-serine (PTH-serine) and the dithiothreitol (DTT) adduct of PTH-serine. As recently reported by Meyer *et al.* (1986), phosphoserine provides exclusively the DTT adduct of PTH-serine. This procedure (a) was monitored with kemptide (LRRASLG, Serva) phosphorylated at its sole serine residue by the catalytic subunit and with unmodified kemptide. Phosphorylated and unphosphorylated kemptide were separated by pH 6.5 electrophoresis. Procedure b used the same approach but was performed on the radioactive tryptic peptides generated from the larger chymotryptic peptides. Tryptic peptides were purified by HPLC on a C18 column. Procedure c was based on the manual Edman degradation. After each step an aliquot was removed for pH 3.5 electrophoresis followed by autoradiography. Inorganic phosphate is released in the step in which the serine-phosphate is encountered.

Polymerization

Polymerization was monitored by standard assays (Kaufmann *et al.*, 1985). Desmin and phosphorylated desmin were dialysed at 0.2 mg/ml from 8 M urea into 10 mM Tris−HCl, pH 7.5, 170 mM NaCl, 10 mM 2-mercaptoethanol, 0.2 mM $MgCl_2$ (20 h at 4°C). Negative staining was with 1% uranylacetate. Micrographs were taken at an original magnification of 20 000.

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References

Burke,B. and Gerace,L. (1986) *Cell*, **44**, 639−652.
Cabral,F. and Gottesmann,M.M. (1979) *J. Biol. Chem.*, **254**, 6203−6206.
Celis,P.M., Larsen,P.M., Fey,S.J. and Celis,A. (1983) *J. Cell Biol.*, **97**, 1429−1434.
Edelman,A.M., Blumenthal,D.K. and Krebs,E.G. (1987) *Annu. Rev. Biochem.*, **56**, 567−613.
Evans,R.M. (1984) *J. Biol. Chem.*, **259**, 5372−5375.
Evans,R.M. and Fink,L.M. (1982) *Cell*, **29**, 43−52.
Fischer,S., Vandekerckhove,J., Ampe,C., Traub,P. and Weber,K. (1986) *Biol. Chem. Hoppe-Seyler*, **367**, 1147−1152.
Fisher,D.Z., Chaudhary,N. and Blobel,G. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6450−6454.
Franke,W.W., Schmid,E., Grund,C. and Geiger,B. (1982) *Cell*, **30**, 103−113.
Franke,W.W., Grund,C., Kuhn,C., Lehto,V. and Virtanen,I. (1984) *Exp. Cell Res.*, **154**, 567−580.
Fraser,B., MacRae,T., Parry,D.A.D. and Suzuki,E. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 1179−1183.
Gard,D.L., Bell,P.B. and Lazarides,E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3894−3898.
Gard,D.L. and Lazarides,E. (1982) *Mol. Cell. Biol.*, **2**, 1104−1114.
Geisler,N. and Weber,K. (1980) *Eur. J. Biochem.*, **111**, 425−433.
Geisler,N. and Weber,K. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4120−4123.
Geisler,N. and Weber,K. (1982) *EMBO J.*, **1**, 1649−1656.

Geisler,N., Kaufmann,E. and Weber,K. (1982) *Cell*, **30**, 277–286.
Geisler,N., Fischer,S., Vandekerckhove,J., Plessmann,U. and Weber,K. (1984) *EMBO J.*, **3**, 2701–2706.
Geisler,N., Kaufmann,E. and Weber,K. (1985) *J. Mol. Biol.*, **182**, 173–177.
Geisler,N., Vandekerckhove,J. and Weber,K. (1987) *FEBS Lett.*, **221**, 403–407.
Georgatos,S.D. and Marchesi,V.T. (1985) *J. Cell Biol.*, **100**, 1955–1961.
Georgatos,S.D. and Blobel,G. (1987a) *J. Cell Biol.*, **105**, 105–115.
Georgatos,S.D. and Blobel,G. (1987b) *J. Cell Biol.*, **105**, 117–125.
Georgatos,S.D., Weber,K., Geisler,N. and Blobel,G. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 6780–6784.
Georges,E., Lefebvre,S. and Mushynski,W.E. (1986) *J. Neurochem.*, **47**, 477–483.
Inagaki,M., Nishi,Y., Nishizawa,K., Matsuyama,M. and Sato,C. (1987) *Nature*, **328**, 649–652.
Ip,W., Hartzer,M.K., Pang,S.Y.Y. and Robson,R.M. (1985) *J. Mol. Biol.*, **183**, 365–375.
Julien,J.-P. and Mushynski,W.E. (1983) *J. Biol. Chem.*, **258**, 4019–4025.
Kaufmann,E., Weber,K. and Geisler,N. (1985) *J. Mol. Biol.*, **185**, 733–742.
Krohne,G., Wolin,S.L., McKeon,F.D., Franke,W.W. and Kirschner,M.W. (1987) *EMBO J.*, **6**, 3801–3808.
Lane,E.B., Goodman,S.L. and Trejdosiewicz,L.K. (1982) *EMBO J.*, **1**, 1365–1372.
McKeon,F.D., Kirschner,M.W. and Caput,D. (1986) *Nature*, **319**, 463–468.
Meyer,H.E., Hoffmann-Posorske,E., Korte,H. and Heilmeyer,L.M.G.,Jr (1986) *FEBS Lett.*, **204**, 61–66.
Nelson,W.J. and Traub,P. (1983) *Mol. Cell Biol.*, **3**, 1146–1156.
O'Connor,C.M., Gard,D.L. and Lazarides,E. (1981) *Cell*, **23**, 135–143.
Quax,W., Egberts,W.V., Hendriks,W., Quax-Jeuken,H. and Bloemendal,H. (1983) *Cell*, **35**, 215–223.
Soellner,P., Quinian,R.A. and Franke,W.W. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7929–7933.
Steinert,P.M., Rice,R.H., Roop,D.R., Trus,B.L. and Steven,A.C. (1983) *Nature*, **302**, 794–800.
Steinert,P.M., Steven,A.C. and Roop,D.R. (1985) *Cell*, **42**, 411–419.
Toelle,H.G., Weber,K. and Osborn,M. (1987) *Eur. J. Cell. Biol.*, **43**, 35–47.
Traub,P. (1985) *Intermediate Filaments*. Springer-Verlag, Berlin.
Traub,P. and Vorgias,C. (1983) *J. Cell Sci.* **63**, 43–67.

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