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New directions

A better understanding of the interactions between microbes and their host cells should ultimately result in better methods of treatment and prevention of disease. Studying these interactions should also further our understanding of fundamental cellular processes, just as the study of the interaction of microbial toxins with eukaryotic cells has been pivotal to understanding transmembrane signalling, protein synthesis and endocytosis. The research presented at this meeting, both in the seminars and in the poster sessions, generated lively discussions that should result in interesting new directions and partnerships for the study of these processes. By this criterion the meeting was a great success, and I hope that it will set a trend for future Bristol-Myers Squibb Symposia.

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Protease-dependent virus tropism and pathogenicity

Yoshiyuki Nagai

Viral tissue tropism in a susceptible host is often determined by virus–receptor interactions. Nevertheless, closely related viruses utilizing the same receptor molecules can display striking differences in tropism, or a virus can cause a localized infection despite the widespread occurrence of the receptor. These events are now explained by another mechanism of tropism, in which host proteases play a major role by activating viral fusion glycoproteins.

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Enveloped animal viruses possess a surface glycoprotein that mediates fusion of the viral envelope with the target cell membrane, thereby enabling the viral genome to enter the cytoplasm. Twenty years ago, the pioneering studies of Ohuchi and Homma[1] as well as those of Scheid and Choppin[2] led to the discovery that the fusion (F) glycoprotein of Sendai virus, a murine pathogen and a member of the Paramyxoviridae, is activated through post-translational endoproteolysis of the inactive precursor glycoprotein by a host cell protease. Subsequent work on other enveloped viruses revealed essentially the same mechanism of proteolytic cleavage for fusion glycoprotein activation and its necessity for viral infectivity. These viruses included Newcastle disease virus (NDV), an avian paramyxovirus[3], influenza A virus of the Orthomyxoviridae[4,5] and human immunodeficiency virus type 1 (HIV-1) of the Retroviridae[6].

In the case of paramyxoviruses, the precursor glycoprotein, termed F_0, is synthesized as a single-chain molecule (Fig. 1). The signal peptide at the amino terminus directs the nascent polypeptide into the secretory pathway and is then removed from the nascent chain. A hydrophobic region near the carboxyl terminus stops the translocation process and anchors the molecule to the lipid bilayer. The F_0 is cleaved at the carboxyl side of a particular arginine to yield the two-chain molecule with the amino-terminal F_2 and the carboxy-terminal F_1 subunits, which remain bound together by a disulfide bond ($F_0 \rightarrow F_2$-S-S-F_1). Cleavage results in the exposure of a long (about 25 amino acids) hydrophobic stretch at the amino terminus of the transmembrane F_1 subunit, which is thought to act as the direct mediator of membrane fusion[7]. Thus the overall structural features of fusion glycoproteins include three hydrophobic domains: the signal peptide, fusion domain and membrane anchor domain. These features are conserved among fusion glycoproteins of different viruses, despite their striking variations in both size and amino acid sequence. Their precursor–product relationships can be denoted as HA (hemagglutinin) $\rightarrow HA_1$-S-S-HA_2 (influenza A virus) and gp160 (Env) $\rightarrow$ gp120–gp41 (HIV-1). The gp120 and gp41 are linked by noncovalent bonds.

Glycoprotein cleavage, tropism and virulence of NDV

The significance for *in vivo* pathogenicity of viral glycoprotein activation by host cell proteases was first demonstrated for NDV (Refs 3, 8). There is a wide variety of strains of this virus, and they differ markedly in tissue tropism and virulence in their hosts, the chicken and chicken embryo. These differences cannot be explained by virus–receptor interactions, because the virus utilizes ubiquitous sialic-acid-containing macromolecules as receptors. The F_0 precursors of avirulent strains are cleaved only in a restricted number of cell types, and only these cell types permit the virus to spread through multiple rounds of replication. Other cell types lack cleavage

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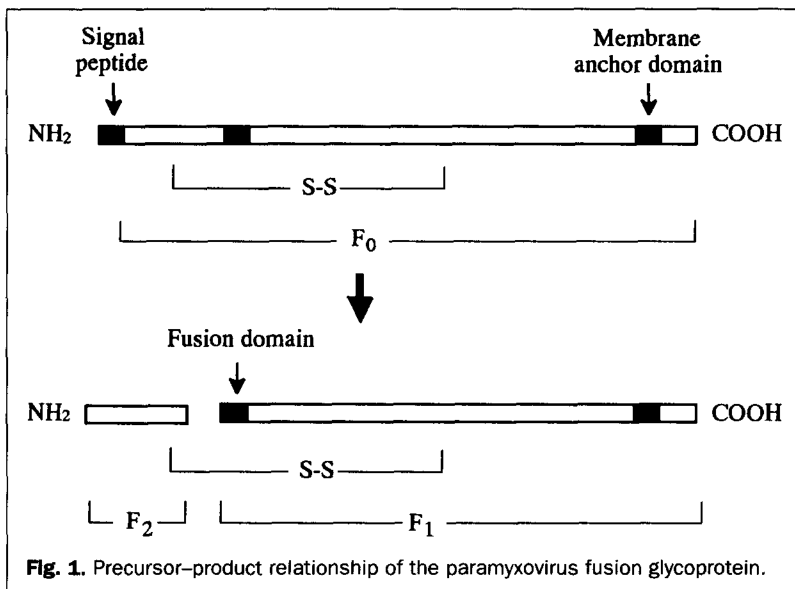

Fig. 1. Precursor–product relationship of the paramyxovirus fusion glycoprotein.

capacity and are nonpermissive for the viruses (Fig. 2). Consequently, they cause a localized infection without producing severe symptoms. In contrast, the F_0 precursors of virulent strains are activated in a broad range of host cells (Fig. 2), and allow the virus to spread rapidly throughout the body, causing a systemic, lethal infection. Moreover, mutants have been isolated from avirulent strains, in which enhanced cleavability is paralleled by an increase in pathogenicity[9]. If trypsin is added to the culture medium of infected cells to promote F_0 cleavage, avirulent strains grow through multiple rounds of replication as efficiently as virulent strains do (Fig. 2). This indicates that F_0 cleavage is virtually the only requirement for full replication of avirulent strains. Glycoprotein cleavability is therefore of prime importance for determining NDV tropism and virulence.

Differences in cleavability appear to be determined mainly by the amino acid sequence at the cleavage site. The F_0 proteins, which are cleaved by ubiquitously occurring proteases, have a cluster of basic residues (RRQK/RR) at this position, whereas the cleavage site is monobasic (QK/RQGR) in those F_0 proteins cleaved by proteases highly specific for particular tissues (Table 1)[10,11]. The former type of cleavage takes place intracellularly at the Golgi apparatus or at the trans-Golgi network[12,13], while the latter type of cleavage is an extracellular event (see below).

Attachment of NDV to host cells is mediated by another glycoprotein, hemagglutinin–neuraminidase (HN). Unlike the F glycoprotein, HN is anchored to the bilayer at its amino terminus. In some avirulent NDV strains, HN is also synthesized as a precursor, HN_0, which is converted into HN by removal of a 9 kDa glycopeptide from its carboxyl terminus[14,15]. Cleavage is again a post-translational event effected by a host protease at a single arginine site, and is necessary for full functioning of the glycoprotein.

The genome of paramyxoviruses is a nonsegmented single-strand RNA of negative sense, which contains a set of six or more genes in tandem, including the ones encoding the two glycoproteins. Nucleotide sequence comparison of many NDV strains has revealed that neither intergenic nor intragenic recombination has occurred and that a limited number of point mutations at the cleavage site must have generated the strains showing different glycoprotein cleavability and pathogenicity characteritics[16,17].

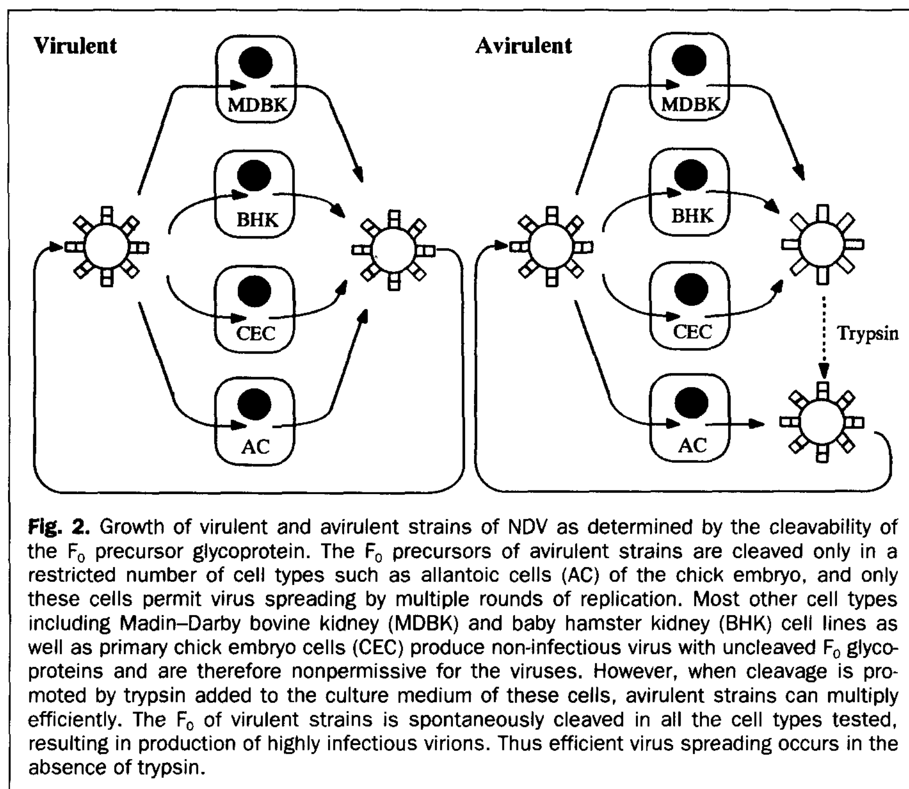

Fig. 2. Growth of virulent and avirulent strains of NDV as determined by the cleavability of the F_0 precursor glycoprotein. The F_0 precursors of avirulent strains are cleaved only in a restricted number of cell types such as allantoic cells (AC) of the chick embryo, and only these cells permit virus spreading by multiple rounds of replication. Most other cell types including Madin–Darby bovine kidney (MDBK) and baby hamster kidney (BHK) cell lines as well as primary chick embryo cells (CEC) produce non-infectious virus with uncleaved F_0 glycoproteins and are therefore nonpermissive for the viruses. However, when cleavage is promoted by trypsin added to the culture medium of these cells, avirulent strains can multiply efficiently. The F_0 of virulent strains is spontaneously cleaved in all the cell types tested, resulting in production of highly infectious virions. Thus efficient virus spreading occurs in the absence of trypsin.

Cleavage-motif-specified differences in influenza A virus tropism

The degree of cleavability of the influenza A virus glycoprotein HA determines whether a virus strain has as its target a wide spectrum of tissues (as virulent avian influenza strains have) or a narrow one (as mammalian and avirulent avian influenza strains have)[18,19]. The cleavage sites of highly cleavable HAs generally consist of multiple basic residues, whereas those of HAs of restricted cleavability consist of a single arginine (Table 1). Thus, influenza A virus presents the same pattern of tropism and pathogenicity as described for NDV. Subsequent analyses of naturally occurring strains, virus mutants adapted *in*

vitro to new host cells, and HA mutants generated by site-directed mutagenesis, have identified several structural indications of cleavability (for details, see Refs 20, 21).

First, the consensus sequence RXK/RR at the cleavage site is the minimum requirement for recognition by the ubiquitous proteases[22–24]. The only exception to this rule so far appears to be A/Chick/Pennsylvania/83 with the KKKR cleavage site (Table 1).

Second, in contrast to NDV (in which point mutations must have generated different cleavage site motifs), in H7 virulent avian influenza virus, a readily cleavable oligobasic motif may have been created by the insertion of a cellular sequence between the HA_1 and HA_2 subunits of an avirulent virus connected by a single arginine (Table 1). The experimental adaptation of H7 seal virus (Table 1) to chicken embryo cells resulted in a similar insertion of three arginine residues before the P1 arginine with the concomitant acquisition of high cleavability of the HA in a broad range of cells and a high degree of pathogenicity for chickens[25]. Likewise, an insertion of a minimum of four arginine residues between P2 threonine and P1 arginine rendered H3 human HA susceptible to the action of the ubiquitous proteases[23]. In contrast, point mutations at the HA_1 carboxyl terminus of this HA, to create the consensus RXK/RR motif, did not result in increased cleavability[23]. A striking example of recombination between viral and cellular RNA is provided by the H7 virus mutant, in which 54 nucleotides from the chicken 28S rRNA have been inserted between P2 threonine and P1 arginine and are translated in frame[26]. The inserted foreign peptide of 18 amino acids, even though they are nonbasic, has rendered the cleavage site with a single arginine susceptible to ubiquitously occurring proteases and made the virus pathogenic for chickens. The increased cleavability in this case seems to be due to bulging of the inserted peptide.

Third, point mutations distant from the cleavage site can also influence HA cleavability and virus host range, as evidenced by mutants adapted to Madin–Darby canine kidney cells[25,27,28]. However, such mutants display enhanced HA cleavage only in the cells to which they have been adapted, and remain nonpathogenic for the chicken.

Table 1. Cleavage site sequence and cleavability of viral glycoprotein precursors

Viral glycoprotein precursor	Cleavage site[a]		Cleavability[b]
Paramyxovirus F_0	P7 P1 ↓		
NDV (avirulent)	GGG**K**QG**R**	LI	N
	GGG**R**QG**R**	LI	N
NDV (virulent)	GG**RR**Q**KR**	FI	Y
	GG**RR**Q**RR**	FI	Y
Sendai virus	AGAPQS**R**	FF	N
Human parainfluenza virus 3	TDP**R**T**KR**	FI	Y
Mumps virus	GS**RR**H**KR**	FA	Y
Simian virus 5	PT**RRRRR**	FA	Y
Measles virus	TS**RR**H**KR**	FA	Y
Respiratory syncytial virus	S**KKRKRR**	FL	Y
Influenza virus A HA	P7 P2 P1		
A/Puerto Rico/8/34(H1)	IPSIQS----**R**	GL	N
A/Japan/305/57(H2)	VPQIES----**R**	GL	N
A/Aichi/2/68(H3)	VPE**K**QT----**R**	GL	N
A/Chick/Pennsylvania/1/83(H5)	VPQ**KKK**----**R**	GL	Y
A/FPV/Rostock/34(H7)	VPEPS**KKR**E**KR**	GL	Y
A/Seal/Massachusetts/1/80(H7)	VPENP**K**T---**R**	GL	N
A/Chick/Germany/49(H10)	VPEVVQG---**R**	GL	N
A/Gull/Maryland/704/77(H13)	VPAISN----**R**	GL	N
Togavirus p62	P7 P1		
Semliki forest virus	NGT**R**H**RR**	SV	Y
Sindbis virus	SSG**R**S**KR**	SV	Y
Flavivirus prM			
Yellow fever virus	**R**S**RR**S**RR**	AI	Y
Japanese encephalitis virus	HS**KR**S**RR**	SV	Y
Coronavirus E2			
Infectious bronchitis virus	GT**RR**F**RR**	ST	Y
Retrovirus Env			
HIV-1	VVQ**R**E**KR**	AV	Y
Visna virus	NLQ**RKKR**	GL	Y
Rous sarcoma virus	TGI**RRKR**	SV	Y
Herpesvirus gB			
Human cytomegalovirus	THN**R**T**KR**	ST	Y
Varicella zoster virus	**R**NT**R**S**RR**	SV	Y

[a]Because of limitations on the permissible number of references, primary publications are not cited. The data are available from EMBL, GenBank and other databases. For influenza HA, dashes are included to give maximum sequence homology, and numbering (P1–P7) is according to the reference sequence of A/Puerto Rico/8/34(H1).
[b]Cleavable (Y) or uncleavable (N) by ubiquitous endoproteases.

Finally, a carbohydrate side chain, in the vicinity of the oligobasic cleavage site of virulent influenza viruses, modulates cleavability possibly by masking the cleavage site[29]. This steric interference can be offset by increasing the basic charge at the cleavage site by the insertion of an additional arginine or lysine there as well as by the removal of the sugar chain[30,31].

Pneumotropism of Sendai virus

The Sendai virus receptor is also made up of ubiquitous sialic acids; however, the virus targets only the respiratory tract. Apparently, a specific trypsin-like protease recognizes the single arginine motif of F_0, facilitates cleavage and allows spread of the virus,

ultimately to cause pneumonia[32]. The absence of significant virus growth in other organs is probably due to the absence of specific virus-activating proteases. This concept of protease-dependent pneumotropism has been substantiated by the following observations. A Sendai virus mutant has been found that displays increased F_0 cleavability owing to two amino acid changes (P2 serine to proline and P1 arginine to lysine); this mutant causes pantropic infections[33]. Another mutant, which has lost the ability to be activated by trypsin owing to an arginine to isoleucine change at P1 (Ref. 34), produces negligible clinical signs and lung lesions when inoculated into mice after activation *in vitro* by chymotrypsin[35]. Nevertheless, this mutant can induce protective immunity against challenge with the wild-type virus, indicating the potential of a protease-activation mutant as a live vaccine[35].

Glycoprotein cleavage and pathogenicity of other viruses

Although enveloped viruses belong to several diverse taxonomic families, the precursor glycoproteins of many possess an oligobasic cleavage site and are readily cleaved in susceptible cells in culture in the absence of supplementary activating proteases (Table 1). This could certainly be an important basis for the systemic and highly viremic infections characteristic of the typically pantropic viruses, such as measles virus, mumps virus and yellow fever virus as well as virulent NDV and virulent avian influenza virus. Respiratory syncytial virus (RSV) and human parainfluenza virus 3 (PIV3) generally cause only localized infection of the respiratory tract, even though their glycoproteins demonstrate high cleavability (Table 1). However, in immunocompromised infants, RSV is multiorgan-tropic, and in cyclophosphamide-treated animals, the virus spreads outside the respiratory tract and into other organs[36] whereas Sendai virus remains localized[37]. PIV3 has a greater predilection to cause pneumonia than does PIV1, which has a single arginine cleavage motif, and even induces a significant level of viremia in humans[38]. Although receptors and other host molecules are important for determining HIV-1 tropism, high cleavability of the gp160 is apparently a precondition for the virus to spread within and between target organs. The same is applicable to many other viruses with an oligobasic cleavage site motif (listed in Table 1). Various structural features have been revealed by cleavage-site-directed mutagenesis of HIV-1 gp160, other retrovirus glycoprotein precursors and paramyxovirus F_0 (Refs 39–41). Among these, the RXK/RR motif again seems to be important as the minimum requirement for recognition by the ubiquitously occurring proteases.

Identification of virus-activating endoproteases

Although protease-mediated virus activation and its importance for the determination of virus tropism and pathogenicity were already recognized in the mid-1970s, it is only recently that the proteases involved have been identified. A virus-activating protease (VAP) catalysing cleavage at a single arginine site was first isolated from the chicken embryo. Subsequently, a different VAP of the same category has been isolated from the rat lung. The oligobasic RXK/RR motif is shared by many cellular proproteins, and the recent progress in identifying their processing enzymes has greatly helped to characterize this type of VAP.

The blood-clotting factor Xa

Avirulent NDV, Sendai virus and mammalian and avirulent avian influenza A viruses are thought to replicate well in the allantoic and amniotic sacs of the chicken embryo because of the availability of a VAP in the fluids filling these sacs[42,43]. By using allantoic and amniotic fluids as starting materials, this VAP has been isolated and purified to homogeneity[44,45]. The VAP activates the viruses by cleaving their F_0 or HA (or the HN_0 of NDV) at a specific single arginine site. The VAP is a calcium-dependent serine protease consisting of two subunits: a catalytic 33 kDa chain and a 23 kDa chain required for calcium binding (Fig. 3, left). The amino-terminal amino acid sequence of each subunit is highly homologous to that of the activated form (FXa) of human or bovine blood-clotting factor X (FX), a member of the vitamin-K-dependent serine proteases (Fig. 3, right). The identity of the VAP with FXa has been confirmed by comparing the primary structure, deduced from the cloned cDNA sequence encoding the VAP, to those of human and bovine FX, and by amino acid sequencing of the FXa isolated from chicken plasma (Fig. 3, right; Ref. 46). FX is generally synthesized in the liver and circulates as one of the plasma protease zymogens. It was therefore an unexpected discovery that not only the liver but also a variety of other chicken embryo tissues, including the allantoic and amniotic cells as well as the kidney, intestine and spleen, express the specific mRNA and the translation product[47]. Also surprising is the finding that active FXa is naturally present in the allantoic and amniotic fluids, since it is usually generated only in the middle phase of the blood-clotting cascade reaction.

Although the role of FXa as a bona fide activator *in ovo* has not yet been fully established, evidence strongly suggesting this has come from the observation that the spread of virus is restricted to tissue surfaces in direct contact with FXa-containing fluids[47]. This finding further supports the notion that cleavage activation is an extracellular event. Indeed, a macromolecular FXa-specific inhibitor, dansylated glutamyl-glycyl-arginine chloromethylketon (dansyl EGR-CMK), which only poorly penetrates cells, can block cleavage activation *in ovo*[44]. This peptidyl CMK mimics the cleavage site of bovine prothrombin, the natural substrate of FXa. The P3 glutamic acid appears to be replaceable with glutamine (Q), suggesting the importance of the carbonyl side chain rather than the negative charge at P3 for substrate recognition[48]. Interestingly, the viral glycoproteins activated in the chicken embryo often have a similar

cleavage site, generalized as E/QXR, where X is G, S or T. This could explain the higher specificity of FXa for virus activation compared with trypsin[44]. However, different cleavage site sequences are seen in some influenza HAs (Table 1), and they are also activated in the allantoic sac. It will be interesting to learn whether they are cleaved by FXa or other enzymes. The amount of FXa required for virus activation *in vitro* is much lower than for trypsin[44]. The high efficiency of FXa appears to be attributable to concentration of the enzyme on the lipid bilayer via Ca^{2+} bound to the γ-carboxyglutamic acids and the first of the two epidermal growth factor-like domains in the amino terminus of the 23 kDa chain.

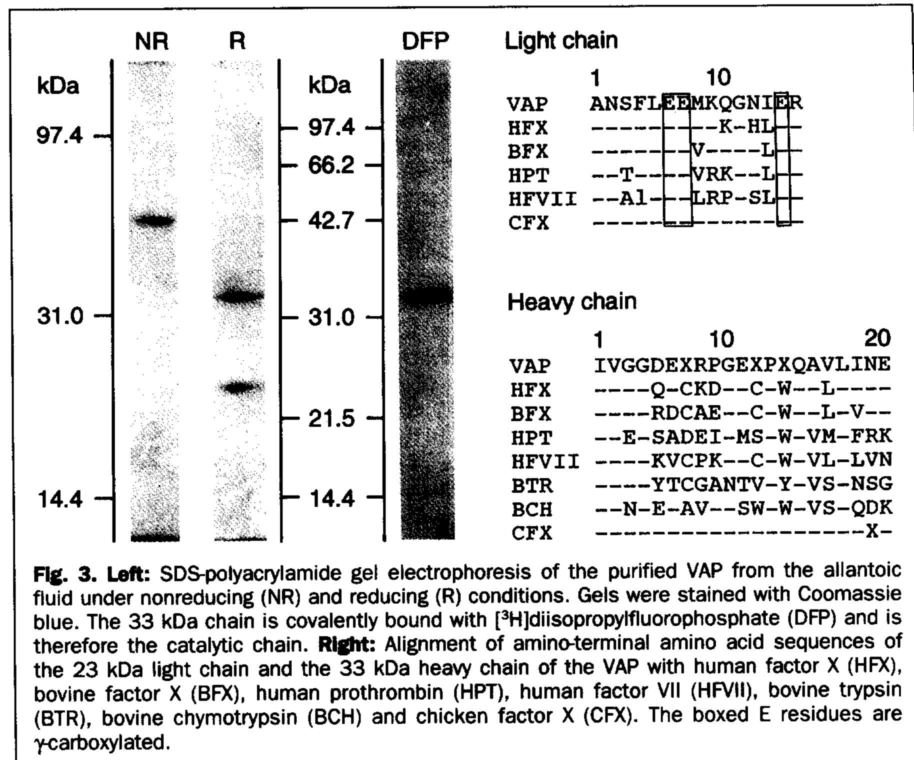

Fig. 3. Left: SDS-polyacrylamide gel electrophoresis of the purified VAP from the allantoic fluid under nonreducing (NR) and reducing (R) conditions. Gels were stained with Coomassie blue. The 33 kDa chain is covalently bound with [^{3}H]diisopropylfluorophosphate (DFP) and is therefore the catalytic chain. **Right:** Alignment of amino-terminal amino acid sequences of the 23 kDa light chain and the 33 kDa heavy chain of the VAP with human factor X (HFX), bovine factor X (BFX), human prothrombin (HPT), human factor VII (HFVII), bovine trypsin (BTR), bovine chymotrypsin (BCH) and chicken factor X (CFX). The boxed E residues are γ-carboxylated.

Tryptase Clara

Rats as well as mice are susceptible to Sendai virus. Tryptase Clara is an arginine-specific serine protease located exclusively in and secreted from Clara cells in the bronchial epithelium of rats[49,50]. The purified enzyme cleaves and activates Sendai virus F_0 and influenza virus HA, both of which feature a single arginine cleavage motif. Calcium is not required for this activation. Moreover, anti-tryptase Clara serum blocks virus activation and replication in the lung, resulting in a reduction in lung lesions and mortality rate[50]. Thus, tryptase Clara is probably the enzyme responsible for the dissemination of Sendai virus and its pathogenicity in the rat lung. Here again, cleavage seems to be an extracellular event that can be inhibited by specific antibodies. The enzyme concentration required for Sendai virus activation *in vitro* is remarkably high (at least 1 μg ml^{-1}; Ref. 50), when compared with chicken FXa (about 30 ng ml^{-1}; Ref. 44). Such a high concentration normally does not appear to occur on the bronchial surface but it seems that following Sendai virus infection, local conditions are modulated to facilitate the activation of progeny viruses by tryptase Clara. It would be interesting to learn how this modulation is brought about.

Furin, a subtilisin-like endoprotease

Oligobasic or dibasic cleavage motifs are frequently found in cellular proproteins and prohormones. The processing at these motifs has therefore always been of great interest and the proteases involved have long been sought for. A landmark in this field of cell biology was the discovery in the yeast *Saccharomyces cerevisiae* of KEX2 (a genetic marker related to killer function) protease, which processes the pro-α mating factor and the pro-killer toxin at the RR and KR sites[51], and shares both sequence and organizational homologies with the prokaryotic subtilisins[52]. Subsequently, several KEX2 homologues in higher eukaryotes have been identified, including furin, PC2 (from a pituitary cell line) and PC3 (formerly called PC1); these constitute a unique family of mammalian subtilisins[53]. Furin is a Golgi membrane-localized endoprotease that is ubiquitously expressed in a variety of tissues and cell lines and is possibly involved in proprotein processing in the constitutive secretory pathway. On the other hand, expression of PC2 and PC3 is restricted to neuroendocrine cells. They are localized to secretory vesicles and considered to be responsible for processing in the regulated secretory pathway. NALM-6, a lymphoid cell line, has a reduced cleaving activity even for F_0 of virulent NDV (Ref. 54). F_0 cleavage in this virus–cell system is stimulated significantly by coexpressing KEX2 in a recombinant vaccinia virus[55]. Using recombinant vaccinia viruses has further revealed that furin is fully capable of cleaving F_0 while PC2 and PC3 are entirely incapable or only partially capable of cleavage (Table 2)[56]. F_0 cleavage by furin results in enhancement of viral infectivity. Neither furin nor PC2 and PC3 can attack the single arginine site of avirulent strains (Table 2). Thus, furin appears to be responsible for cleavage at oligobasic sites or the consensus RXK/RR site.

There have been extensive attempts to isolate from Madin–Darby bovine kidney (MDBK) cells an endoprotease that activates the influenza fowl plaque virus (FPV) HA (Ref. 57). Although purification to homogeneity has not been accomplished, the protease has been sufficiently concentrated to establish its identity with furin by a specific antiserum and by affinity labelling with a radioactive peptidyl CMK that mimics the cleavage site. It has also been shown that HA

Table 2. Processing of cellular and viral proproteins by subtilisin-related proteinases

Proprotein	Processing motif (P6 to P1)	Cell line	Cleavage[a] by				Refs
			KEX2	Furin	PC2	PC3	
Pro-von Willebrand factor	SHRSKR	COS-1	NT	+	NT	NT	59, 60
Pro-β nerve growth factor	THRSKR	BSC-40	+	+	NT	NT	61
Pro-complement 3	AARRRR	COS-1	NT	+	NT	NT	62
Proalbumin	RGVFRR	COS-1	+[b]	+	NT	NT	63
Prorenin	DVFTKR	CHO	NT	–	NT	–	63
		GH_4C_1	NT	–	NT	+	63
Prorenin M2R^{-4}	DVRTKR	CHO	NT	+	NT	–	63
NDV virulent F_0	GRRQRR	NALM-6	+	+	–	+/–	55, 56
	GRRQKR	NALM-6	+	+	–	+/–	55, 56
NDV avirulent F_0	GGRQGR	NALM-6	–	–	–	–	55, 56
	GGKQGR	NALM-6	–	–	–	–	55, 56

[a]Proteinases and proproteins were coexpressed in the respective cells. +, fully cleavable; –, uncleavable; +/–, partially cleavable; NT, not tested.
[b]*In vitro* assay.

cleavage increases in cells where the HA and furin are coexpressed by vaccinia virus vectors. Furthermore, cleavage-site-specific decanoyl-REKR-CMK, which is able to penetrate cells, inhibits intracellular cleavage activation of HA (Ref. 57). A very similar pattern of increased glycoprotein cleavage by coexpressed furin and activation inhibition by decanoyl-REKR-CMK has been demonstrated for HIV-1 (Ref. 58). Although its identity has not yet been established, an endoprotease of the same category that cleaves the F_0 of virulent NDV has been isolated from Golgi membranes of rat liver cells[54].

The specificity of mammalian subtilisins for cellular proteins has also been examined by similar coexpression studies[59–63]. When expressed in cells that probably lack the regulated secretory pathway, for example COS-1, BSC-40 and CHO cells, furin correctly processes most of the substrates (Table 2). Except for proalbumin, these substrates possess an arginine at the P4 position (in proalbumin, an arginine occupies P6). Prorenin, which has a dibasic motif but no P4 arginine, cannot be processed by furin. However, furin can cleave a mutant prorenin in which the P4 phenylalanine has been replaced by arginine (Table 2). Taken together, as for viral precursors, the RXK/RR motif generally seems to be decisive for furin-mediated processing in the constitutive secretory pathway. Studies on the KR motif of prorenin suggest that PC3 operates in endocrine GH_4C_1 cells but not in CHO cells (Table 2). This apparent cell specificity of PC3, and probably also of PC2, may account for their inability to process virulent NDV F_0. However, these enzymes can process pro-opiomelanocortin correctly at KR and RR sites even in the constitutive secretory pathway[64]. The RXK/RR motif with P4 arginine, rather than the simpler KR and RR, thus appears to be a prerequisite for furin-mediated processing in this secretory pathway and, fittingly, it is widely conserved among viral, cellular and plasma proproteins.

Concluding remarks

Virus spread and tissue tropism depend strongly on the proper match between the cleavability of the viral glycoprotein by an endoprotease and the availability of the protease in the host. The structure of the cleavage site is of prime importance for determining whether the virus is activated in a wide variety of tissues or in specific tissue types. With its ability to recognize the single arginine cleavage site, FXa became the first VAP to be identified, followed by tryptase Clara. The VAPs in this category thus differ depending on the host and/or tissue type. It would be interesting to identify influenza-virus-activating proteases in the human respiratory tract. It should be noted that besides proteases of host origin, those from coinfecting bacteria may also be involved in virus activation[65–67].

The ubiquitously occurring VAP that recognizes the oligobasic motif has been identified as furin, but the question remains whether it is the only protease of its type. In this context, another member of the mammalian subtilisins, PACE-4, is worthy of investigation because of its widespread tissue distribution[68]. The PK cleavage site of the pantropic Sendai virus[33] appears to be unique; the complementary protease that acts on this motif remains to be identified.

Although our current knowledge of protease-mediated virus activation and its significance for viral pathogenicity is still incomplete, it has already stimulated a variety of new approaches to virus disease control. For instance, the therapeutic potential of specific protease inhibitors is being tested, with peptidyl CMKs specific for cleavage site sequences taking the lead[44,57,58]. Avirulent NDV strains with a single arginine motif for protease recognition have already been used routinely for protection of birds against the virulent viruses with a highly cleavable oligobasic motif. This highlights the potential of attenuated live vaccines made by cleavage-site-directed mutagenesis. For example, if the oligobasic cleavage sites can be

made monobasic, measles virus and mumps virus could be attenuated for use as live vaccines. Human influenza A virus is similar to murine Sendai virus in that both possess a single arginine motif and target the respiratory tract. In view of the potential of a protease-activation mutant of Sendai virus as a live vaccine (see above and Ref. 35), it would be worth engineering similar mutants of influenza virus that have a cleavage site susceptible to enzymes other than arginine-specific ones. The technology to create these cleavage-site-specific mutants has become fully available for many viruses including influenza A virus, but not yet for others, such as the paramyxoviruses. VAPs undoubtedly are not solely involved in virus activation. So, what is the physiological significance of ectopic FX/FXa expression in the chicken embryo? What physiological roles does tryptase Clara play in the rat lung? These are new issues raised by the research on VAPs and well worthy of investigation.

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References

1 Homma, M. and Ohuchi, M. (1973) *J. Virol.* 12, 1457–1465
2 Scheid, A. and Choppin, P.W. (1974) *Virology* 57, 475–490
3 Nagai, Y., Klenk, H-D. and Rott, R. (1976) *Virology* 72, 494–508
4 Klenk, H-D. *et al.* (1975) *Virology* 68, 426–439
5 Lazarowitz, S.G. and Choppin, P.W. (1975) *Virology* 68, 440–454
6 McCune, J.M. *et al.* (1988) *Cell* 53, 55–67
7 Gething, M.J., White, J.M. and Waterfield, M.D. (1978) *Proc. Natl Acad. Sci. USA* 75, 2737–2740
8 Nagai, Y. *et al.* (1979) *J. Gen. Virol.* 45, 263–272
9 Garten, W. *et al.* (1980) *J. Gen. Virol.* 50, 135–147
10 Toyoda, T. *et al.* (1987) *Virology* 157, 242–247
11 Gickman, R.L. *et al.* (1988) *J. Virol.* 62, 354–356
12 Nagai, Y., Ogura, H. and Klenk, H-D. (1976) *Virology* 69, 523–538
13 Morrison, T.G., Ward, L.J. and Semerjian, A. (1985) *J. Virol.* 53, 851–857
14 Nagai, Y. and Klenk, H-D. (1977) *Virology* 77, 125–134
15 Schuy, W. *et al.* (1984) *Virus Res.* 1, 415–426
16 Sakaguchi, T. *et al.* (1989) *Virology* 169, 260–272
17 Toyoda, T. *et al.* (1989) *Virology* 169, 273–282
18 Bosch, F.X. *et al.* (1979) *Virology* 95, 197–207
19 Bosch, F.X. *et al.* (1981) *Virology* 113, 725–735
20 Rott, R. (1992) *Vet. Microbiol.* 33, 303–310
21 Klenk, H-D. *et al.* in *Virus Strategies* (Doerfler, W., ed.), Verlag Chemie (in press)
22 Vey, M. *et al.* (1992) *Virology* 188, 408–413
23 Ohuchi, R. *et al.* (1991) *J. Virol.* 65, 3530–3537
24 Kawaoka, Y. and Webster, R.G. (1988) *Proc. Natl Acad. Sci. USA* 85, 324–328
25 Li, S. *et al.* (1990) *J. Virol.* 64, 3297–3303
26 Katchikian, D., Orlich, M. and Rott, R. (1989) *Nature* 340, 156–157
27 Rott, R. *et al.* (1984) *EMBO J.* 3, 3328–3332
28 Orlich, M. *et al.* (1990) *Virology* 176, 531–538
29 Kawaoka, Y., Naeve, C.W. and Webster, R.G. (1984) *Virology* 139, 303–316
30 Kawaoka, Y. and Webster, R.G. (1989) *J. Virol.* 63, 3296–3300
31 Ohuchi, M. *et al.* (1989) *Virology* 168, 274–280
32 Tashiro, M. and Homma, M. (1983) *Arch. Virol.* 77, 127–137
33 Tashiro, M. *et al.* (1988) *Virology* 165, 577–583
34 Itoh, M., Shibuta, H. and Homma, M. (1987) *J. Gen. Virol.* 68, 2939–2944
35 Tashiro, M. and Homma, M. (1985) *J. Virol.* 53, 228–234
36 McIntosh, K. and Chanock, R.M. (1990) in *Virology* (2nd edn) (Fields, B.N. *et al.*, eds), pp. 1045–1072, Raven Press
37 Blandford, G. (1975) *Immunology* 28, 871–883
38 Chanock, R.M. and McIntosh, K. (1990) in *Virology* (2nd edn) (Fields, B.N. *et al.*, eds), pp. 963–988, Raven Press
39 Bosch, V. and Pawlita, M. (1990) *J. Virol.* 64, 2337–2344
40 Dubay, J.W. *et al.* (1991) in *Immunobiology of Proteins and Peptides* (Atassi, M.Z., ed.), pp. 39–46, Plenum Press
41 Paterson, R.G. *et al.* (1989) *J. Virol.* 63, 1293–1301
42 Muramatsu, M. and Homma, M. (1980) *Microbiol. Immunol.* 24, 113–122
43 Appleyard, G. and Davis, G.B. (1982) *J. Gen. Virol.* 64, 813–823
44 Gotoh, B. *et al.* (1990) *EMBO J.* 9, 4189–4195
45 Gotoh, B. *et al.* (1992) *FEBS Lett.* 296, 274–278
46 Suzuki, H. *et al.* (1991) *FEBS Lett.* 283, 281–285
47 Ogasawara, T. *et al.* (1992) *EMBO J.* 11, 467–472
48 Kettner, C. and Shaw, E. (1981) *Thromb. Res.* 22, 645–652
49 Kido, H. *et al.* (1992) *J. Biol. Chem.* 267, 13573–13579
50 Tashiro, M. *et al.* (1992) *J. Virol.* 66, 7211–7216
51 Julius, D. *et al.* (1984) *Cell* 37, 1075–1089
52 Fuller, R.S., Sterne, R.E. and Thorner, J. (1988) *Annu. Rev. Physiol.* 50, 345–362
53 Barr, P.J. (1991) *Cell* 66, 1–3
54 Sakaguchi, T. *et al.* (1991) *Virology* 184, 504–512
55 Nagai, Y., Inocencio, N.M. and Gotoh, B. (1991) *Behring Inst. Mitt.* 89, 35–45
56 Gotoh, B. *et al.* (1992) *J. Virol.* 66, 6391–6397
57 Stieneke-Gröber, A. *et al.* (1992) *EMBO J.* 11, 2407–2414
58 Hallenberger, S. *et al.* (1992) *Nature* 360, 358–361
59 van deVen, W.J.M. *et al.* (1990) *Mol. Biol. Rep.* 14, 265–275
60 Wise, R.J. *et al.* (1990) *Proc. Natl Acad. Sci. USA* 87, 9378–9382
61 Bresnahan, P.A. *et al.* (1990) *J. Cell Biol.* 111, 2851–2859
62 Misumi, Y. *et al.* (1991) *J. Biol. Chem.* 266, 16954–16959
63 Hosaka, M. *et al.* (1991) *J. Biol. Chem.* 266, 12127–12130
64 Thomas, L. *et al.* (1991) *Proc. Natl Acad. Sci. USA* 88, 5297–5301
65 Tashiro, M. *et al.* (1987) *Nature* 325, 536–537
66 Akaike, T. *et al.* (1989) *J. Virol.* 63, 2252–2259
67 Scheiblauer, H. *et al.* (1992) *J. Infect. Dis.* 166, 783–791
68 Kiefer, M.C. *et al.* (1991) *DNA Cell Biol.* 10, 757–769

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