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Molecular Signaling in the Regulation of Mucins

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Abstract Mucins are large, highly glycosylated proteins involved in the protection of epithelia. The 20 different mucins show a diverse and highly regulated distribution among different epithelia. Most of the studies on mucin regulation done to date have been on the membrane mucins MUC1 and MUC4 and the gel-forming mucins. Multiple mechanisms have been implicated in that regulation, including examples at the transcriptional, transcript stabilization and post-translational levels. Several signaling pathways have been demonstrated to be involved, most frequently the canonical Erk MAP kinase pathway, but also the cytokine-JAK-STAT pathway and TGFβ-SMAD pathways. Diversity in Erk signaling is achieved through multiple activation mechanisms and multiple downstream transcriptional factors that are affected. Given the still limited amount of information available on regulation of most of the mucins, other mechanisms and pathways are likely to be uncovered in the future. J. Cell. Biochem. 102: 1103–1116, 2007. © 2007 Wiley-Liss, Inc.

Key words: mucin; regulation; transcript; Erk; epithelia; carcinomas

Mucins are large, highly *O*-glycosylated proteins which provide protection for epithelial surfaces. Although they are frequently treated as a gene family, most mucins represent simply a collection of molecules with similar characteristics. The two major features are the large fraction of serine and threonine residues which are *O*-glycosylated and the presence of tandem repeats [Carraway, 2000]. Some 20 different human mucins (Table I) have been described, primarily from sequence data that provide the first characterization. Note that human mucins are designated in capitals, as in MUC1, while rodent mucins are indicated as Muc. Mucins are generally divided into two classes, secreted and membrane, based both on sequence data and biochemical studies. This classification is a bit misleading, as some membrane mucins can be cleaved within the cell and secreted [Komatsu et al., 2002]. Perhaps the best functional classification is into three categories: membrane, which have a transmembrane sequence; gel-forming, secreted mucins found in mucus gels; and other. Functionally, gel-forming mucins provide hydration, lubrication, transport, and protection mechanisms for mucosa. They are produced primarily by specialized cells, goblet cells embedded in epithelia or submucosal gland cells [Ali and Pearson, 2007]. Membrane mucins not only provide the ultimate barrier for the epithelial surface but also stimulate additional protective mechanisms, for example, to promote cell survival [Komatsu et al., 2001; Raina et al., 2004], by their involvement in cell signaling [Carraway et al., 2003]. Membrane mucins are produced by many epithelia. Much of the attention directed toward mucins results from their roles in diseases of the epithelia in which production of the mucins is altered, including carcinomas, which are derived from epithelial cells.

BIOSYNTHESIS

The synthesis of mucins follows the general scheme of all secreted and cell surface glycoproteins. Transcripts, which for most mucins are extremely large, are translated on endoplasmic reticulum-associated ribosomes and threaded through a channel into the ER lumen. *N*-glycosides are added co-translationally, and the signal sequence that specifies ER binding is removed by proteolysis. The glycoproteins next undergo a "copy-editing" step to eliminate misfolded proteins that involves deglucosylation/

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TABLE I. Human Mucin Genes; Search Conducted Through NCBI Genomic Biology, Human Genome (5/2007)

Human mucin	Approved name	Ref. Seq. ID's	Chromosome	Gene ID
MUC1	Mucin 1, cell surface associated	NM_002456	1q22	4582
MUC2	Mucin 2, oligomeric mucus/gel-forming	NM_002457	11p15.5	4583
MUC3/17	Mucin 17, cell surface associated	NM_001040105	7q22	140453
MUC3A	Mucin 3A, cell surface associated	AF113616	7q22	4584
MUC3B	Mucin 3B, cell surface associated	AJ291390	7q22	57876
MUC4	Mucin 4, cell surface associated	NM_018406	3q29	4585
MUC5AC	Mucin 5AC, oligomeric mucus/gel-forming	XM_001130382	11p15.5	4586
MUC5B	Mucin 5B, oligomeric mucus/gel-forming	XM_001126093	11p15.5	727897
MUC6	Mucin 6, oligomeric mucus/gel-forming	XM_290540	11p15.5	4588
MUC7	Mucin 7, secreted	NM_152291	4q13.3	4589
MUC8	Mucin 8	U14383	12q24.3	4590
MUC9	Oviductal glycoprotein 1, 120 kDa (mucin 9, oviductin)	NM_002557	1p13.2	5016
MUC10	Record discontinued			386748
MUC11	Withdrawn and substituted by MUC12			10071
MUC12	Mucin 12, cell surface associated	XM_379904	7q22	10071
MUC13	Mucin 13, cell surface associated	NM_033049	3q21.2	566671
MUC14	EMCN, endomucin	NM_016242	4q22.1	51705
MUC15	Mucin 15, cell surface associated	NM_145650	11p14.3	143662
MUC16	Mucin 16, cell surface associated	NM_024690	19p13.2	94025
MUC18	MCAM, melanoma cell adhesion molecule	X68264	11q23.3	4162
MUC19	Mucin 19, oligomeric	AY236870	12q12	283463
MUC20	Mucin 20, cell surface associated	NM_152673	3q29	200958

glucosylation and proteosomal degradation [Helenius and Aebi, 2004]. The proteosome may also provide a regulatory mechanism for some mucins to prevent their overexpression (see section on Post-Translational Regulation of Muc4). *O*-glycosylation and further processing of the *N*-glycosides occurs during the transit of the mucins to the cell surface. In the case of the gel-forming mucins, disulfide formation between subunits and packaging into granules also happens during this transit [Perez-Vilar and Hill, 1999]. The soluble form of at least one membrane mucin, Muc4, is also packaged into secretory granules in selected tissues [Rossi et al., 1996; Arango et al., 2001]. Although membrane mucins are frequently pictured as perpendicular to the membrane, it is clear that this orientation is not possible in the transit compartments responsible for glycosylation, since the mucin polypeptides are much longer than the glycosyltransferases which must glycosylate them. In order to account for the extensive glycosylation along the mucin polypeptides and the relatively rigid structure resulting from that glycosylation, the membrane mucins must be parallel and proximal to the membrane as they are being glycosylated. This requirement may explain why most, if not all, membrane mucins undergo a cleavage into two subunits early in their transit to the cell surface [Sheng et al., 1990; Hilkens et al., 1992]. Perhaps they assume a more perpendicular orientation after they reach the cell surface.

DISTRIBUTION

Mucins exhibit a highly ordered tissue distribution, indicating a tight regulation of their expression. Some, such as the membrane mucins MUC1 and MUC4, are present in multiple tissues. Others have more limited expression, such as MUC2 predominantly in the intestine. Expression is also developmentally regulated, usually arising at a specific developmental stage and continuing in the adult [Reid and Harris, 1998]. An interesting case occurs with MUC4 in the skin, which is present in the embryo before cornification, but not in the adult [Zhang et al., 2006]. The airway appears to produce the largest variety of mucins [Rose and Voynow, 2006] and provides examples of cell-specific expression. MUC5AC is secreted by goblet cells of the airway luminal epithelium, while MUC5B is secreted by airway glandular epithelium [Rose and Voynow, 2006]. Carcinomas, which are derived from epithelial cells, frequently exhibit an altered expression of mucins compared to their normal counterparts [Hollingsworth and Swanson, 2004]. For example, overexpression of MUC1 is a characteristic of most breast carcinomas [Gendler, 2001]. MUC16 was originally recognized by an antibody developed for diagnosis of

ovarian cancer [Yin and Lloyd, 2001]. Dysregulation of mucin expression also frequently accompanies inflammatory responses [Voynow et al., 2006].

One problem with assessing mucin expression in various tissues is the variability of the methods used. Much of the early work was done by analyzing transcripts by Northern blotting or in situ hybridization. However, mucin protein levels do not always correspond to the transcript levels. A noteworthy case is rat Muc4 in the mammary gland, which is post-translationally downregulated in the virgin gland but not in the lactating gland [Price-Schiavi et al., 1998]. Analysis of mucin protein levels can be difficult, as mucin antibodies are notoriously fickle, particularly for immunohistochemistry. Antibodies made against tandem repeat sequences frequently do not recognize mature forms of the mucin, in which these sequences are heavily glycosylated [Burchell et al., 2001]. Antibodies against other domains may be similarly masked by fixation during tissue preparation.

TRANSCRIPTIONAL REGULATION

Promoter Regions of Mucin Genes

Much of the work on mucin regulation has been directed toward transcriptional control. The diversity of gene expression patterns described above can be ascribed to two aspects of transcriptional regulation: specific, unique promoter sequences in the MUC genes and differential, tissue specific expression and regulation of transcriptional factors. Previous reviews have provided extensive descriptions of promoter sequences [Van Seuningen et al., 2001; Andrianifahanana et al., 2005], which have served to identify a plethora of specific transcription factors involved in MUC gene regulation (Table II). Most of these have been characterized by analyses of their specific interactions with the defined promoter elements. What is needed now is a better understanding of how these transcription factors are regulated and activated.

TABLE II. Transcription Factors Involved in Mucin Gene Regulation

Transcription factor	Mucin gene regulated
Sp1 family	*MUC1*, *MUC2*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC6*
NFκB	*MUC1*, *MUC2*, *MUC5B*
CDX family	*MUC2*, *MUC4*
GATA family	*MUC4*, *MUC5B*
STAT	*MUC1*
PEA3	Rat *Muc4*

Differentiation Factors

Mucins are the products of differentiated epithelia. Consequently, it is not surprising that mucin production is affected by factors that promote differentiation. What is sometimes difficult to discern is whether changes in mucins result only from cellular changes or also from increases in gene expression within those cells. For example, airway epithelia respond to irritants by increasing the number of goblet cells which secrete the gel-forming mucin MUC5AC [Rose and Voynow, 2006]. Chronic goblet cell hyperplasia also occurs in chronic airway diseases such as asthma. Thus, the resulting increase in mucin production can be partially explained by the increase in the terminally differentiated goblet cells. However, studies in cell culture models also suggest that differentiation factors can directly affect mucin gene expression.

The primary differentiation factor studied has been retinoic acid (Table III), which acts via specific nuclear receptors RAR and RXR to activate gene transcription [Leid et al., 1992]. Retinoic acid contributions to airway cell differentiation depend on its integration with a complex set of environmental factors, including the extracellular matrix [Moghal and Neel, 1998]. Normal human bronchial epithelial cells undergo mucosecretory differentiation when grown in the presence of retinoic acid on collagen gel, which is necessary for the induction of the retinoic acid receptor. An important effect of the retinoic acid is to repress signaling through the canonical mitogenic pathway Raf-Erk (Fig. 1). Inhibition of either the upstream receptor tyrosine kinase, the epidermal growth factor receptor (EGFR), or the Erk kinase MEK could replace the collagen requirement for retinoic acid-dependent differentiation. What other pathways may be involved is unclear, but studies in some other systems have shown the ratio of the activities of two MAP kinases, p38 and Erk, to be an indicator of differentiation [Nebreda and Porras, 2000].

In the case of the membrane mucin MUC4, retinoic acid induced increased gene expression in the pancreatic tumor cell via its receptor RARα [Choudhury et al., 2000]. Surprisingly,

TABLE III. Regulatory Factors Involved in Mucin Expression

Mucin	Factor	Effect	Tissue	Pathway	Mechanism	References
MUC1						
	IFNγ	↑	Breast	NFκb/stat1	Transcription	Lagow and Carson [2002]
	IFNγ	↑	Hematopoetic	?	Transcription	Reddy et al. [2003]
	IL7	↑	Human T cells	?	Transcription	Vasir et al. [2005]
	Indole 3-carbinol	↓	Breast	?	Transc./product.	Lee et al. [2004]
	Neutrophil elastase	↑	Lung	SP1	Transcription	Kuwahara et al. [2005]
	TNFα	↑	Uterus	NFκb	Transcription	Thathiah et al. [2004]
	TNFα	↑	Nasal epithel.	TNFR	Transcription	Shirasaki et al. [2003]
MUC2						
	Butyrate	↑	Colon tumor	Erk	Transcription	Hatayama et al. [2007]
	EGF	↑	Airway	SP1	Transcription	Perrais et al. [2002]
	Galectin3	↑	Colon tumor	?	Transc./product.	Dudas et al. [2002]
	IL1	↑	Airway		Transcription	Kim YD
	IL4, 13, TNFα	↑	Colon tumor	Mapk	Transcription	Iwashita et al. [2003]
	PMA	↑	Colon tumor	Ras/MEK	Transcription	Lee et al. [2002]
	Roxythromycin	↓	Intestine	NFκb	Transcription	Kim et al. [2004]
	TNFα	↓	Colon tumor	JNK, NFκb	Transcription	Ahn et al. [2005]
	Vasoactive peptide	↑	Colon	Erk/p38	Transcription	Hokari et al. [2005]
MUC4						
	CFTR	↓	Pancreas	?	Transc./post-trans.	Singh et al. [2007]
	IGF	↑	Rat mammary	Erk	Transc./product.	Zhu et al. [2000]
	IL4, IL9	↑	Lung	Jak3	Transcription	Damera et al. [2006]
	Interferon-γ and RA	↑	Pancreas	stat1/RAR	Transcription	Adrianifahanana [2005]
	Neutrophil elastase	↑	Airway	?	Transcription	Fischer et al. [2003]
	Retinoic acid (RA)	↑	Pancreas	RAR/TGFβ2	Transcription	Choudhury et al. [2000]
	TGFβ	↓	Pancreas	Smad2/4	Transcription	Jonckheere et al. [2004]
	TGFβ	↓	Uterus	?	Transc./product.	Idris [2000]
	TGFβ	↓	Mammary	SMAD	Post-translation	Price-Schiavi [1998, 2000]; Soto [2003]
MUC5AC						
	Dexamethasone	↓	Lung tumor	?	Transc./product.	Lu et al. [2005]
	Dexamethasone	↑	Primary lung	?	Translation	Lu et al. [2005]
	Dexamethasone	↓	Lung	GRE	Transcription	Chen et al. [2006]
	EGF	↑	Airway	SP1	Transcription	Perrais et al. [2002]
	EGF	↑	Gall bladder	MAP/Erk	Transc./product.	Finzi et al. [2006]
	EGF	↑	Airway	EGFR activ.	Transc./product.	Casalino-Matsuda et al. [2006]
	EGFR	↑	Airway	MAPK/Akt	Transc./product.	Kitazaki et al. [2005]
	HAT	↑	Airway	EGFR/Erk	Transcription	Chokki et al. [2004, 2005]
	IL13	↑	Airway	TGFα, EGF	Transcription	Zheng [2007]
	IL13	↑	Airway	Differentiation	Multiple levels	Yasuo et al. [2006]
	IL17	↑	Airway	Erk/MAPK	Production	Inoue et al. [2006]
	IL1β	↑	Airway	Cox2/PKA	Production	Gray et al. [2004]
	IL1β	↑	Airway	Erk/p38	Transcription	Song et al. [2003]
	Neutrophil elastase	↑	Lung tumor	ROS/NQO1	Multiple levels	Zheng [2007]
	Neutrophil elastase	↑	Airway	NFκb/MAPK	Transcription	Song et al., 2005b
	Neutrophil elastase	↑	Airway	ROS/PKC	Transc./product.	Shao and Nadel [2005]
	Neutrophil elastase	↑	Airway	ROS	mRNA stability	Fischer and Voynow [2002]
	Neutrophil elastase	↑	Airway	TGFα/EGF	Production	Kohri et al. [2002]
	NO	↑	Airway	PKC	Transcription	Song et al. [2007]
	PMA	↑	Airway	Ras/MEK/Sp1	Transc./product.	Hewson et al. [2004]
	ProstaglandinE2	↑	Airway	?	Transc./product.	Kook Kim et al. [2006]
	TGFα	↑	Airway	e-cadh./EGF	Production	Kim et al. [2005]
	TGFβ2 + IL13	↑	Airway	?	Transc./product.	Chu et al. [2004]
	TNFα	↑	Airway	IKKβ	Transc./product.	Lora et al. [2005]
	TNFα	↑	Nasal epithel.	Erk	Transcription	Young Kim et al. [2004]
	TNFα	↑	Airway	Erk/p38	Transcription	Song et al. [2003]
MUC5B						
	Neutrophil elastase	↑	Lung tumor	ROS/NQO1	Multiple levels	Zheng [2007]
	IL6, IL17	↑	Airway	Jak/Stat/Erk	Transcription	Chen et al. [2003]
	PMA	↑	Airway	Ras/Erk	Transcription	Yuan-Chen Wu et al. [2007]
	RA	↑	Airway	?	Transcription	Chen et al. [2001]

the retinoic acid appeared to act via an intermediate TGFβ, which has been shown in other systems to repress Muc4 expression by a post-translational [Price-Schiavi et al., 2000] or transcriptional [Idris and Carraway, 2000] mechanism. Anti-TGFβ blocked both TGFβ- and retinoic acid-induced expression of the MUC4 gene in the pancreatic tumor cells. Moreover, RARα antagonists inhibited the upregulation of both TGFβ-2 and MUC4 transcripts. Thus, TGFβ appears to be able to act on MUC4 by a number of mechanisms depending on cell context.

Phorbol esters serve as both differentiation factors and tumor promoters, depending on cell context, frequently by activating protein kinase C (PKC) isoforms. Phorbol 12-myristate 13-acetate (PMA) increases the transcription of

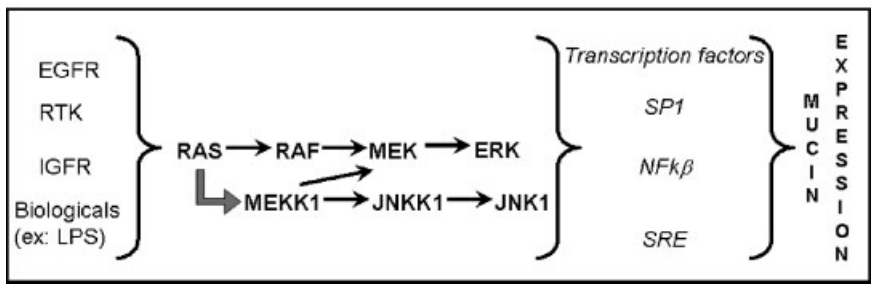

Fig. 1. Variety of inputs and outputs of MAPK signaling pathways involved in mucin regulation.

MUC2 in HM3 colon cancer cells, acting on a 5′-flanking promoter region containing Sp1 and NFκB sites [Lee et al., 2002]. Analyses using pharmacological inhibitors and dominant negative effectors implicated activation of the Ras-Erk pathway via PKC. NFκB was also directly implicated. Activated Erk participates in the regulation of several different mucin genes (Table III, Fig. 1), involving different upstream activators and downstream effectors, representing the variety of mechanisms by which this pathway may participate in cellular regulation.

Butyrate acts as a differentiation agent for a number of cell types, including those of the colon, in which it is produced by anaerobic bacteria. Treatment of the human colon cancer cell line LS174T with butyrate induces mucin expression, increases histone H3 acetylation and activates MUC2 production by stimulating *MUC2* gene expression [Hatayama et al., 2007]. Cell cycle arrest, but not apoptosis, accompanied this treatment. Moreover, inhibition of MEK blocked MUC2 production, implicating the Erk signaling pathway (Fig. 1). These results suggest an activation of the *MUC2* gene by butyrate that involves inhibition of histone deacetylation and Erk phosphorylation.

Cytokines

As products of the immune system, cytokines are defined by their immune functions. Type 1 cytokines are associated with a cellular immune response and include interferon-γ, tumor necrosis factor, interleukins (IL) 1, 2, and 12. Type 2 cytokines are associated with a humoral immune response and include interleukins 4–6, 9, 10, and 13. Cytokines bind to specific receptors to activate associated tyrosine kinases that initiate downstream signaling. The most common effect of cytokines is activation of the JAK/STAT pathway, in which the tyrosine kinase JAK phosphorylates the transcription factor STAT to induce its dimerization. The STAT dimer then migrates to the nucleus to regulate transcription. Interferon-γ has been shown to promote expression of MUC1 in a number of cell types, including ovarian carcinoma [Clark et al., 1994], hematopoietic [Reddy et al., 2003], and prostate tumor [O'Connor et al., 2005] cells, apparently by STAT binding to a site in the MUC1 promoter [Gaemers et al., 2001]. Tumor necrosis factor-α (TNFα) is also able to enhance MUC1 in multiple cell types. In prostate tumor cells this effect has been shown to be synergistic with interferon-γ [O'Connor et al., 2005].

MUC4 is also regulated transcriptionally by interferon-γ [Andrianifahanana et al., 2007]. Studies in pancreatic cancer cells show that the *MUC4* gene expression is delayed relative to a second gene, that for interferon regulatory factor-1, requiring an increase in *STAT1* gene expression. Chromatin immunoprecipitation assays indicate that STAT1 binds directly to interferon-γ-activation sites in the *MUC4* promoter. Interestingly, interferon-γ acts synergistically with the differentiation factor retinoic acid [Andrianifahanana et al., 2006]. Apparently, this synergy requires shifts in the downstream pathways, since interferon-γ represses the retinoic acid induction of TGFβ and retinoic acid inhibits the upregulation of STAT1 by interferon-γ. Such findings emphasize the ability of tumors to evolve and select new mechanisms to promote growth and survival characteristics during progression. Interferon-γ has no effect on Muc4 expression in rat mammary epithelial cells, but can repress post-translational regulation of Muc4 by TGFβ by

inducing SMAD7 [Soto et al., 2003]. Thus, the interferon can act at multiple levels in regulating mucin expression depending on cell context.

Gel-forming mucins are also regulated by type 1 cytokines, as exemplified by studies on MUC2 and MUC5AC in airway epithelial and tumor cells [Kim et al., 2002; Koo et al., 2002; Song et al., 2003]. In normal human nasal epithelial cells *MUC5AC* gene expression is promoted by the type 1 inflammatory cytokines TNFα and IL-1β via pathways that require both Erk and p38 MAP kinases [Song et al., 2003]. The cyclic AMP response element in the *MUC5AC* promoter has been implicated, with its phosphorylation and activation mediated by the mitogen- and stress-activated kinase MSK1, downstream of Erk and p38 MAPK. IL-1β in NCI-H292 cells, a model system for airway diseases, induced both *MUC5AC* and *MUC2* gene expression by a similar mechanism, requiring both Erk and p38 MAPKs [Kim et al., 2002]. These in turn activated cyclooxygenase-2 to produce prostaglandin E2. Expression of the mucin genes was blocked by COX-2 inhibitors and induced by PE2. Upregulation of the gene expression can also be blocked by retinoic acid inhibitors, suggesting a requirement for differentiation in these cells for mucin production [Koo et al., 2002].

Type 2 cytokines, such as IL-4 and IL-13, have been implicated in goblet cell metaplasia and in the regulation of mucins produced by goblet cells, MUC2 and MUC5AC [Andrianifahanana et al., 2006]. IL-4 and IL-13 act via a common receptor IL-4R to activate STAT6. Whether they are directly involved in transcriptional activation of the gel-forming mucin genes is somewhat controversial because of differences observed between different cell and animal systems and the question of effects on goblet cell regulation versus mucin gene regulation. For example, IL-4 induced *MUC2* gene expression in NCI-H292 cells and *Muc5* gene expression in mouse airway epithelial cells in vivo [Dabbagh et al., 1999]. In contrast, IL-4 inhibited the expression of *MUC5AC* and *MUC5B* in normal human tracheobronchial cells [Jayawickreme et al., 1999]. In guinea pig tracheal epithelial cell cultures, treatment with IL-13, but not IL-4, increased the number of goblet cells and the amount of secreted MUC5AC [Kondo et al., 2002]. However, *MUC5AC* gene expression was decreased by IL-13 in human nasal epithelial cells even though expression of *MUC2* and *MUC8* genes were increased [Kim et al., 2002]. Unfortunately, we know too little about the pathways and mechanisms involved in the effects of these cytokines to be able to understand how these different effects arise.

Bacterial Products

Since a major function of mucins is to protect epithelia from infection, it is not surprising that bacterial products can alter mucin gene expression (Table IV). Much of the work has been done on airway epithelial cells and has provided some interesting mechanistic observations. For example, both Gram-positive and Gram-negative bacteria induce expression of the *MUC2* gene via the Ras-Raf-MEK-Erk canonical mitogenic pathway, even though they act through different cell surface receptors [McNamara and Basbaum, 2001]. Gram-negative *Pseudomonas aeruginosa* can alter *MUC2* gene expression via two cell surface components: lipopolysaccharide (LPS) and flagellin. Both of these induce an Src-dependent activation of Ras, leading to downstream activation of Erk and p90rsk. p90rsk phosphorylation of the NFκB inhibitory complex frees it to bind to a promoter sequence on the *MUC2* gene. LPS activates the Ras pathway via a toll-like receptor. Flagellin induces this gene activation by binding to the glycolipid asialoGM1 to stimulate the release of ATP from the cells [McNamara and Basbaum, 2001]. ATP then binds to a cell surface G protein-coupled receptor to activate phospholipase C (PLC). PLC cleaves phosphatides to produce diacylglycerol and IP_3, which mobilize calcium from intracellular stores. DAG plus calcium can then activate PKC, leading to stimulation of multiple downstream pathways, including the Src-dependent activation of Ras that stimulates *MUC2* gene activation [McNamara and Basbaum, 2001].

MUC5AC gene expression can be induced by P6 outer membrane protein of *Haemophilus influenzae* binding to cell surface toll-like receptors [Chen et al., 2004]. P38 and NFκB are stimulated through TAK1. p38-activated AP1 and NFκB can then bind to the *MCU5AC* promoter to induce its transcription.

The active factor in Gram-positive bacteria is lipoteichoic acid, which binds to platelet-activating factor receptor, a G protein-coupled receptor [McNamara and Basbaum, 2001]. The GPCR transactivates the EGFR by stimulating proteolytic release of an EGFR ligand,

TABLE IV. Biological Agents Regulating Mucin Expression

Mucin	Agent	Effect	Cell/tissue	Pathway	Mechanism	References
MUC2						
	Bile acids	↑	Colon carcinoma	AP1	Transc./produc.	Song et al. [2005a]
	Haemophilus inf.	↑	Various epithelia	NFκb/Smad	Transcription	Jono et al. [2002]
	H. pylori	↑	Gastric epithelia	?	?	Babu et al. [2006]
	LPS	↑	Nasal epithelia	?	Transcription	Ishinaga et al. [2005]
	Shigella	↑	Intestine	TNFα, PKC, Erk	Production	Radhakrishnan et al. [2007]
MUC3						
	Amyloid A3	↑	Intestine	?	Transc./produc.	Larson et al. [2003]
	Lactobacillus	↑	Intestine	?	Transc./produc.	Mack et al. [2003]
	Listeriolysin	↑	Intestinal	?	Transc./produc.	Lievin-Le Moal et al. [2002]
	Rhinovirus	↑	Airway surface	?	Transc./produc.	Inoue et al. [2006]
MUC4						
	Bile acid	↑	Oesophageal	HNFA1	Transc./produc.	Piessen et al. [2007]
	Bile acid	↑	Oesophageal	PI3K	transcription	Mariette et al. [2004]
	Listeriolysin	↑	Intestinal	?	Transcription	Lievin-Le Moal et al. [2002]
	Wood smoke	↑	Airway	?	Transcription	Bhattacharyya et al. [2004]
MUC5AC						
	Acrolein	↑	Airway	MMP/EGFR	Transcription	Deshmukh et al. [2005]
	Complement	↑	Airway	?	Production	Dillard et al. [2007]
	Listeriolysin	↑	Intestinal	?	Production	Lievin-Le Moal et al. [2002]
	LPS	↑	Intestinal	IL8 signaling	Transc./produc.	Smirnova et al. [2003]
	Pseudomonas	↑	Airway	EGF/MAPK	Transc./produc.	Kohri et al. [2002]
	RSV	↑	Airway	stat1	?	Hashimoto et al. [2005]
	Rhinovirus	↑	Airway surface	SRC, MAPK	Transcription	Inoue et al. [2006]
	Shigella	↑	Intestine	TNFα, PKC, Erk	Production	Radhakrishnan et al. [2007]
	S. Pneum	↑	Airway	IKK/Erk	Transcription	Ha et al. [2007]
	Smoke	↑	Airway	EGFR	Transc./produc.	Baginski et al. [2006]
	Smoke	↑	Airway	TACE/EGFR	Transcription	Shao et al. [2004]
	Wood smoke	↑	Airway	?	Transcription	Bhattacharyya et al. [2004]
MUC5B						
	LPS	↑	Intestinal	IL8 signaling	Transc./produc.	Smirnova et al. [2003]
	Otitis media	↑	Middle ear	?	Transcription	Elsheikh and Mahfouz [2006]
	Rhinovirus	↑	Airway surface	?	Transcription	Inoue et al. [2006]
MUC6						
	H. pylori	↑	Gastric epithelia	?	?	Xia et al. [2004]
	H. pylori	↓	Gastric epithelia	?	?	Babu et al. [2006]
	Rhinovirus	↑	Airway	?	Transcription	Inoue et al. [2006]

heparin-binding EGF, from its cell surface precursor. The precursor is cleaved by the metalloprotease ADAM10. Activated EGFR stimulates Ras by coupling through an adapter complex with the Ras activator SOS [Carraway and Carraway, 1995].

Interestingly, the LPS from the gram-negative, periodontopathic bacteria Porphyromonas gingivalis represses mucin synthesis and increases apoptosis in salivary gland acinar cells [Somiany and Slomiany, 2002]. Both p38 and Erk MAP kinases were involved and stimulated the production of NO through NO synthase. The data on the MAPKs show that opposite responses on mucin expression can be obtained through the same pathways in different cell types.

Growth Factors and Receptors

Cell surface receptors play a critical role in sensing environmental changes and responding. The EGFR acts as an important damage sensor in the airway to regulate mucin gene expression [Burgel and Nadel, 2004]. Two mechanisms appear to be important in activation of the receptor. First, expression of the EGFR is reported to be increased by airway damage [Burgel et al., 2000]. Second, EGF, the ligand for the EGFR, is present in airway luminal fluid, but segregated from the receptor by the epithelial polarity barrier [Vermeer et al., 2006]. Damage to the epithelium breaks the polarity barrier and permits the ligand-receptor association necessary for activation of the receptor. Airway fluid also contains neuregulin [Vermeer et al., 2003], the ligand for two other members of the EGFR family, ErbB3 and ErbB4. Interestingly, the membrane mucin Muc4 acts as an intramembrane ligand for ErbB2, localizing it in the apical membrane, which can segregate it from forming active heterodimeric complexes with the other receptors until the polarity barrier is broken [Ramsauer et al., 2003, 2006; Carraway and Carraway, 2007].

EGFR can be activated by other mechanisms, including transactivation by GPCR via activation of proteases that release EGFR ligands from their membrane precursors, as noted above. Multiple proteases have been reported to be involved in ligand production, either directly or indirectly, including neutrophil elastase, tumor necrosis factor alpha-converting enzyme (TACE) and tissue kallikrein [Nadel, 2007]. A key aspect is how these proteases are activated. Oxidant damage, such as that induced by cigarette smoke, stimulates the cellular production of reactive oxygen species (ROS) via NADPH and dual oxidase-1. ROS activate the protease TACE to cleave the precursor for TGFα to release it as an active ligand for EGFR [Nadel, 2007]. Activated EGFR stimulates *MUC5AC* gene expression via the Erk pathway and NFκB [Nadel and Burgel, 2001].

Other ligand-receptor combinations can also stimulate mucin synthesis via the Erk pathway. Insulin-like growth factor (IGF), but not EGF, will promote the expression of the *Muc4* gene in rat mammary epithelial cells [Zhu et al., 2000], probably mimicking what happens in the mammary gland prior to pregnancy. IGF treatment activates the Erk pathway, and the induction of Muc4 is prevented by MEK inhibitors or dominant negative MEK. The *Muc4* gene is regulated in part by the PEA3 transcription factor [Perez et al., 2003]. PEA3 is activated by signaling through two different MAPK pathways, Erk and JNK.

POST-TRANSCRIPTIONAL REGULATION

Transcript Stabilization

Even if a gene is expressed to make a transcript, there are multiple mechanisms by which production of the protein and its appearance at the cell surface, whether in the membrane or secreted, can be abrogated. Rapid transcript turnover is one of the earliest control mechanisms after transcription and is regulated by specific sequences of the transcript which bind stabilizing proteins. Neutrophils play an important role in inflammatory processes. For two mucins neutrophil elastase has been shown to increase mucin levels in airway cells by transcript stabilization: MUC5AC [Fischer and Voynow, 2002] and MUC4 [Fischer et al., 2003]. In the case of MUC5AC, the transcript stability required the production of ROS. How these then are involved in transcript stabilization is yet unclear. However, ROS generated through NADP(H):quinine oxidoreductase I by neutrophil elastase have also been shown to influence *MUC5AC* gene expression [Zheng et al., 2007]. Thus, there are multiple mechanisms by which the elastase can regulate mucins.

Post-Translational Regulation

Muc4 is produced in abundant amounts in the rat mammary gland and secreted into milk [Rossi et al., 1996]. In contrast, about 100-fold less Muc4 is produced by the gland of virgin animals, even though the transcript levels are about the same in the virgin and lactating animals [Price-Schiavi et al., 1998]. Temporal analyses indicate that the change in Muc4 levels occurs around mid-pregnancy. Studies of isolated mammary epithelial cells showed a marked inhibition of Muc4 production by TGFβ [Price-Schiavi et al., 1998, 2000], consistent with observations by others that TGFβ levels in the mammary gland fall in mid-pregnant animals [Ewan et al., 2002]. Muc4 levels are also high in rat mammary tumors [Rossi et al., 1996] and some human breast cancers [Komatsu et al., 1999], consistent with a loss of responsiveness of many tumors to TGFβ [Derynck et al., 2001; Wilson et al., 2005]. Analyses of the pathway involved in the TGFβ effect on Muc4 in rat mammary cells showed an activation of SMAD2; SMAD2 antisense blocked the ability of TGFβ to inhibit Muc4 production [Soto et al., 2003]. Interestingly, interferon-γ was also able to block the TGFβ inhibition of Muc4 production, though it had no affect acting alone on the mammary cells. Interferon-γ was shown to act by increasing the levels of the inhibitory SMAD7 in the mammary cells [Soto et al., 2003].

Muc4 is synthesized as a high M_r precursor that is cleaved in the endoplasmic reticulum to its two subunits [Sheng et al., 1990]. Pulse-chase labeling studies in rat mammary epithelial cells indicated that TGFβ represses this cleavage [Price-Schiavi et al., 2000]. Thus, the synthesis of Muc4 is being blocked in the endoplasmic reticulum. One potential mechanism for this effect is that the precursor fails to pass the "copy-editing" step in processing and is shunted into the proteosome for degradation. This mechanism is supported by observations that proteosome inhibitors can inhibit the ability of TGFβ to repress Muc4 production.

Moreover, inhibition of proteosome activity represses precursor cleavage to the two subunits.

TGFβ may be a general physiological inhibitor of Muc4 production. In the rat cornea Muc4 is found only in the most superficial layers of the stratified epithelium [Swan et al., 2002]. Treatment of stratified cultures of rat corneal epithelial cells with TGFβ inhibited Muc4 production and resulted in accumulation of aggregates of Muc4 in the basal and medial cell layers, indicating the Muc4 is being produced in all of the cell layers, but is degraded by the proteosome in the basal and medial cell layers. In the normal cornea Muc4 production is likely being suppressed by TGFβ; both the growth factor and its ligand are primarily present in the more basal layers of the stratified epithelium [Tuli et al., 2006].

The other site at which Muc4 regulation is very important is the uterus [McNeer et al., 1998]. Muc4 expression at the surface of the uterine epithelium is high in the virgin rat, but disappears during pregnancy just before implantation. Studies in isolated uterine epithelial cells indicate that the key factor in the repression of Muc4 is TGFβ [Idris and Carraway, 2000]. Although the loss of Muc4 in vivo is due to changes in the balance of progesterone and estrogen [McNeer et al., 1998], these hormones have no effect on Muc4 production in the cultured uterine epithelial cells [Idris and Carraway, 2000]. Instead, the inhibition appears to be due to a paracrine effect, as shown by co-cultures of uterine fibroblasts and epithelial cells. Progesterone acts on the fibroblasts to produce TGFβ, which then inhibits Muc4 production by the epithelial cells. Interestingly, both transcript and protein levels are repressed in cells treated by TGFβ [Idris and Carraway, 2000], suggesting that the growth factor is acting at multiple levels in these cells.

PERSPECTIVE

Undoubtedly, we have only begun to tap the regulatory mechanisms responsible for the highly diverse patterns of mucin expression, whether tissue or cellular. Not surprisingly, much of the effort has been directed toward disease conditions, particularly diseases of the airway and carcinomas. Carcinomas have been investigated both as models for normal epithelia and to understand the altered expression of mucins that frequently accompany neoplasia. In either case one caveat that must be recognized is the heterogeneity of tumors. One surprising feature of this research to date is the prevalence of the Erk pathway in mucin regulation. One suspects that this general mechanism for regulation is frequently discovered because of the availability of reagents for its investigation and that other mechanisms responsible for fine-tuning regulation in different cell types will be uncovered as this research advances.

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