Coronavirus infection of polarized epithelial cells

John W.A. Rossen, Marian C. Horzinek and Peter J.M. Rottier

Epithelial cells are the first host cells to be infected by incoming coronaviruses. Recent observations *in vitro* show that coronaviruses are released from a specific side of these polarized cells, and this polarized release might be important for the spread of the infection *in vivo*. Mechanisms for the directional sorting of coronaviruses might be similar to those governing the polar release of secretory proteins.

J.W.A. Rossen, M.C. Horzinek and P.J.M Rottier are in the Virology Divn of the Dept of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands.*
**tel: +31 30 2532485, fax: +31 30 2536723, e-mail: P.Rottier@vetmic.dgk.ruu.nl*

Epithelial cells form highly organized sheets that line the body cavities of higher eukaryotes and are the first barrier against infection. Their plasma membranes are organized to form an apical face, directed towards the external milieu, and a basolateral face, oriented towards the internal environment. Tight junctions with neighbouring cells separate the two faces and not only confine their components, but also restrict intercellular diffusion.

Viruses can enter epithelial cells or be released from them through either membrane face (for a review, see Ref. 1). Polarized virus entry is often a result of the polarized distribution of the viral receptor, as shown for vesicular stomatitis virus and simian virus 40 (Ref. 1). The presence of the receptor only on the basolateral surface significantly hinders infection. Although not the only determinant, polarized virus release can influence viral spread. Basolateral release allows the infection of underlying tissues and the spread of virus in the blood leading to systemic infection, while apical release from epithelial cells can limit viral spread by preventing the infection of other cell types. For example, parainfluenza viruses, which cause a localized infection of the respiratory tract in humans, are released by budding through the apical membrane[2]. Similarly, Sendai virus, which is exclusively pneumotropic in mice, also buds from the apical surface of epithelial cells, while a mutant Sendai virus that could infect multiple cell types was found to bud through both the apical and basolateral faces[3].

Coronaviruses are enveloped, positive-strand RNA viruses infecting humans, animals and birds. While each virus has a narrow host range, the consequences of infection range from subclinical to lethal, and symptoms include respiratory and enteric disease (most commonly), as well as hepatitis, peritonitis and encephalomyelitis[4]. However, primary replication is often limited to epithelial cells of the respiratory or gastrointestinal tracts (Table 1). A better insight into the interaction of coronaviruses with these cells is important for understanding their pathogenesis. This article summarizes current knowledge and presents recent results from our studies *in vitro*.

Coronavirus infection of epithelia

Coronaviruses are assembled in the intermediate or budding compartment[5,6], which is located between the rough endoplasmic reticulum and the Golgi complex. The viral particles are transported in vesicles through the secretory pathway to the plasma membrane, where they are released by exocytosis[7]. Virions can also be released by lysis of dying cells. Obviously, directional release is significant to coronavirus pathogenesis only when the epithelial layer stays intact. Although infected cells are extruded from the epithelial layer and replaced by new ones[8–12], it is only after excessive cell loss that the monolayer disintegrates[13].

In neonates, the replacement of (infected) epithelial cells is slower and more cells are infected than in adults; consequently, epithelial lesions are more severe[14,15]. This is consistent with the observation that neonates are often fully susceptible to a coronavirus that does not affect older animals[4,14–17]. Age-dependent sensitivity to a virus can also be determined by other factors. For example, a receptor protein for transmissible gastroenteritis virus (TGEV) that is restricted to the villous enterocytes of newborn animals has been found recently[15]. Another factor that may contribute to the high sensitivity of neonates is the lack of natural killer activity in their intraepithelial lymphocytes[15].

Release of mouse hepatitis virus (MHV) *in vivo*

MHV is the best-studied coronavirus. It has many strains, which differ in tropism. Viruses of the enterotropic biotype (such as MHV-Y) infect the intestinal mucosa, with little infection of other tissues (see Ref. 17 and references contained). Infection of 2–3 week old mice results in mild intestinal lesions with minimal alteration of the mucosal architecture, and the virus does not spread in the blood, as it does in 4–7 d old mice[16]. Virus is shed in faeces[14], which suggests that MHV-Y is released apically from enterocytes into the gut lumen.

In contrast, the respiratory strains MHV-A59 and MHV-JHM disseminate to other organs after initial replication in the upper respiratory mucosa. Possibly, virus release occurs from the basolateral face or by cell lysis. Some evidence for

basolateral release comes from MHV-JHM infection of the central nervous system (CNS). Initial infection of ependymal cells appears to be crucial to subsequent pathogenesis and to virus spread within the CNS. Infected ependymal cells maintain their normal appearance, but subependymal tissues become infected a little later. Virus release from the polarized ependymal cells apparently occurs basolaterally, although apical release may also occur[18]. Similarly, MHV-JHM causes retinal disease in mice by initial replication in the retinal epithelium followed by infection of the underlying retinal layers while the epithelium is still intact[19]. These results are consistent with the basolateral release of this MHV strain.

Table 1. Coronavirus–host relationships

Virus[a]	Host	Initial replication	Disease
BCV	Cow	Respiratory tract[b]	Respiratory disease, enteritis
CCV	Dog	Intestine	Enteritis
FCoV	Cat	Intestine	Enteritis, polyserositis, granulomatous infections in many organs
HCV	Human	Respiratory tract	Respiratory disease[c]
HEV	Pig	Upper respiratory tract	Vomiting and wasting disease, encephalomyelitis
IBV	Chicken	Respiratory tract	Respiratory disease, nephritis, gonaditis
MHV^{ent}	Mouse	Intestine	Enteritis[d]
MHV^{res}	Mouse	Respiratory tract	Enteritis, hepatitis, encephalomyelitis, vasculitis
PEDV	Pig	Intestine	Enteritis
PRCV	Pig	Nasal mucosa[e]	Subclinical, respiratory disease
RbCV	Rabbit	Intestine	Enteritis
RCV, SDAV	Rat	Nasal mucosa	Respiratory disease, adenitis
TCV	Turkey	Intestine	Enteritis
TGEV	Pig	Intestine[f]	Enteritis

[a]Abbreviations: BCV, bovine coronavirus; CCV, canine coronavirus; FCoV, feline coronavirus; HCV, human coronavirus; HEV, porcine haemagglutinating encephalomyelitis virus; IBV, avian infectious bronchitis virus; MHV^{ent}, mouse hepatitis virus (enteric strains, e.g. MHV-Y); MHV^{res}, mouse hepatitis virus (respiratory strains, e.g. MHV-A59 and MHV-JHM); PEDV, porcine epidemic diarrhoea virus; PRCV, porcine respiratory coronavirus; RbCV, rabbit coronavirus; RCV, rat coronavirus; SDAV, sialodacryoadenitis virus; TCV, turkey coronavirus; TGEV, porcine transmissible gastroenteritis virus.
[b]BCV probably replicates in the respiratory tract before infecting the gut.
[c]HCV might also be involved in enteric and neurological diseases in humans.
[d]In young mice, enterotropic MHV strains can also cause encephalitis.
[e]PRCV also replicates in enterocytes.
[f]TGEV also replicates in the respiratory tract.

Polar release of other coronaviruses

There is evidence for the polar release of other coronaviruses from epithelial cells. In outbreaks of infectious bronchitis of chickens, the infections are initially respiratory, but viraemia and nephritis can follow. As the tracheal epithelium usually remains intact, this suggests that basolateral release is occurring (see Ref. 20 and references contained), which is consistent with the interpretation that infectious bronchitis virus (IBV) is released from the lateral membranes of chicken kidney epithelial cells in the absence of cell lysis[21].

Electron-microscopic analysis of isolated ileum and jejunum loops from 7 d old pigs infected with TGEV showed many virus particles in the lumen, especially in proximity to the microvilli, before the cells started to degenerate, which suggests that apical release is occurring[13]. Viral particles are frequently found near the apical plasmalemma of bronchiolar cells in animals infected with TGEV or porcine respiratory coronavirus (PRCV)[12]. Furthermore, human coronavirus has been observed to be released apically, and virions are shed from intact epithelia into the nasal cavity[22]. For bovine coronavirus, there is no clear polarity of virus release, and virions are found not only free in the intestinal lumen (often lining the apical plasma membrane of normal-looking epithelial cells), but also in intercellular spaces[8].

Release of coronaviruses *in vitro*

We have recently started to study the sorting mechanism of viruses that bud intracellularly in model systems of coronavirus-infected cells grown on filter supports[23,24]. Filter-grown epithelial cells differentiate to become fully polarized. Under these conditions, the apical and basolateral plasma-membrane faces of the cells can be accessed separately.

Using MHV-A59 and TGEV in murine and porcine epithelial cells, respectively, we found that infection with either virus only became established when the virus was added to the apical side of the cells. For TGEV, this is because the viral receptor is only present on the apical membrane. By determining the amount of viral proteins and infectious particles present in the apical and basolateral media, we found that TGEV was released preferentially from the apical membrane domain. In contrast, MHV was released mainly from the basolateral membranes. These results have been confirmed by electron microscopy: TGEV particles were seen attached to apical plasma-membrane domains, and no particles were detected in the spaces between the filter and the cells, nor in the intercellular spaces. In contrast, MHV particles accumulated in these spaces and were rarely observed attached to the apical membrane (Fig. 1).

Conclusions and perspectives

Our studies *in vitro* show that MHV and TGEV both enter epithelial cells preferentially through the apical membrane domain. This is not

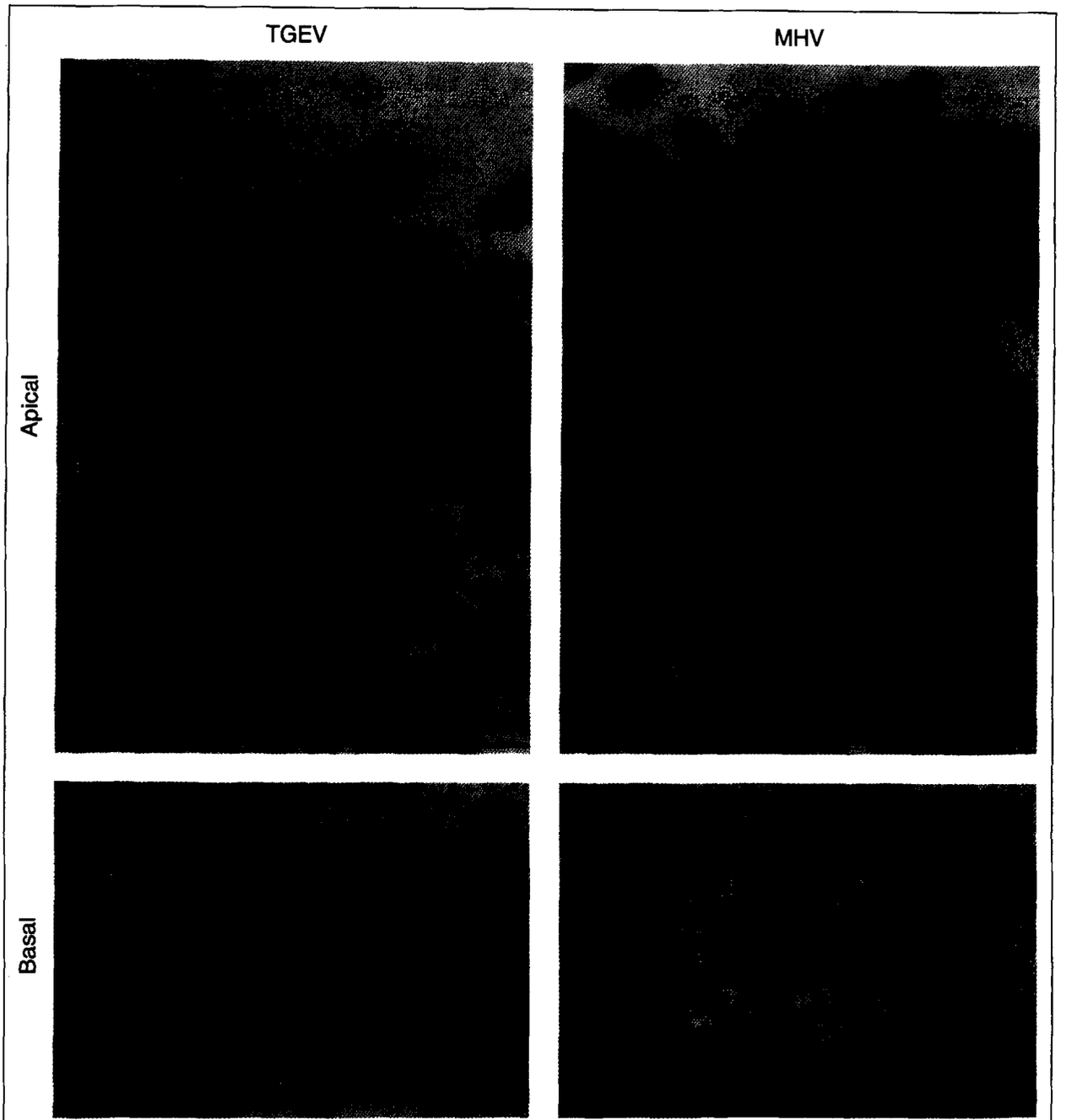

Fig. 1. Release of transmissible gastroenteritis virus (TGEV) and mouse hepatitis virus (MHV) from polarized epithelial cells. Porcine and murine epithelial cells were grown on filter supports and infected with TGEV and MHV-A59, respectively. TGEV virions (arrows) are seen adsorbed to the apical membrane; they are not found between the basal membrane and the filter, which is visible at the bottom of the lower panels. In contrast, many MHV virions (arrowheads) accumulate in the intercellular spaces just beneath the tight junctions (asterisk) and between the basal membrane and the filter. No MHV particles appear at the apical membrane. 'V' represents a vesicle filled with MHV particles. Scale bar = 0.25 μm in all panels.

surprising – it is the surface that the virus first encounters during natural infection. The combined *in vivo* and *in vitro* data indicate that the release of coronaviruses from epithelial cells is polarized. Furthermore, coronaviruses that spread beyond the epithelial mucosa seem to be released basolaterally (for example, MHV and IBV), whereas viruses that remain confined to the respiratory or intestinal epithelium are released apically (for example, human coronavirus, TGEV and PRCV). Although it would be premature to draw general conclusions from a few studies, the polarized release of coronaviruses from epithelial cells is consistent with the differences in pathology.

An intriguing question remains: how do epithelial cells sort intracellularly budding viruses to different membrane domains? Conceivably, virus-containing vesicles are sorted by the same mechanisms that govern the polar release of secretory proteins. As yet, these mechanisms remain unknown, but the involvement of one or more vesicle-membrane

proteins carrying specific targeting information is likely[25] (Fig. 2). For example, the targeting of lysosomal enzymes to lysosomes occurs via a membrane-bound receptor that recognizes the mannose-6-phosphate modification of the enzyme molecules[26]. The mannose-6-phosphate receptor has been shown to colocalize with lysosomal enzymes along the secretory pathway to the apical membrane in the osteoclast, a polarized cell that secretes large amounts of lysosomal enzymes into an apical cavity[27].

If it is assumed that the TGEV receptor is directly targeted to the apical membrane, then the receptor might also guide TGEV virions to this domain. In MHV infection, another host protein (but not the virus receptor) might be used. Alternatively, the viral receptor might be transported to the basolateral membrane and subsequently transcytosed to the apical domain.

It is possible that the coronaviral spike protein is involved in sorting; not only is the spike protein incorporated into virions, but a fraction of the spike protein molecules is also independently transported to the plasma membrane, and virions might be cosorted into specific vesicles together with free spike protein. In this way, the spike protein might confer specific targeting information to the vesicles. We are currently studying the transport of independently expressed coronavirus spike proteins in epithelial cells to see if these proteins are responsible for the differences between the pathways followed by TGEV and MHV. The materials and methods available promise to answer questions that arise at the interface between molecular cell biology and viral pathogenesis.

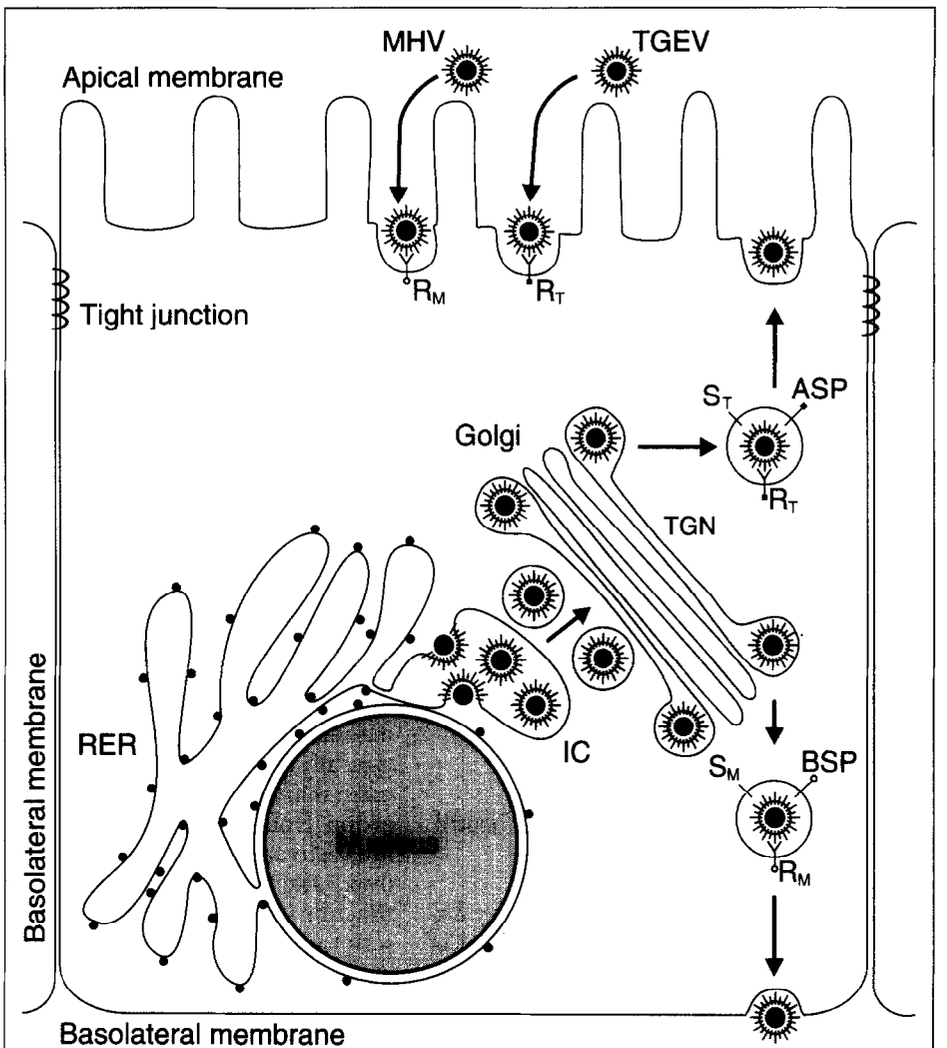

Fig. 2. Possible mechanisms for the directional release of transmissible gastroenteritis virus (TGEV) and mouse hepatitis virus (MHV). Both TGEV and MHV enter the epithelial cell through the apical plasma-membrane domain. Progeny virions are assembled in the intermediate compartment (IC) and transported to the *trans*-Golgi network (TGN), where the sorting of TGEV and MHV virions into different vesicles is thought to occur. Vesicles containing TGEV and MHV particles are transported to the apical and basolateral plasma-membrane domain, respectively. The vesicles might have acquired their targeting specificity by the co-incorporation into their membranes of molecules that are specifically targeted, and the viral spike protein and the virus receptor might be such molecules. Alternatively, apical- or basolateral-sorting proteins mediating targeted delivery of the viruses might be specifically cosorted into the vesicles. Abbreviations: RER, rough endoplasmic reticulum; ASP, apical-sorting protein; BSP, basolateral-sorting protein; R_M, MHV receptor; R_T, TGEV receptor; S_M, MHV spike protein; S_T, TGEV spike protein.

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Questions to be answered

- Are coronaviruses sorted into specific transport vesicles?
- What are the biological implications of the polarized release of viruses?
- By what mechanism(s) is the targeted delivery of coronaviruses to apical or basolateral spaces achieved?
- What relevance do *in vitro* model systems have for understanding natural infection?

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Information processing in bacteria

Two-component Signal Transduction
edited by James A. Hoch and Thomas J. Silhavy

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$79.00/£59.50 hbk (xvi + 488 pages)
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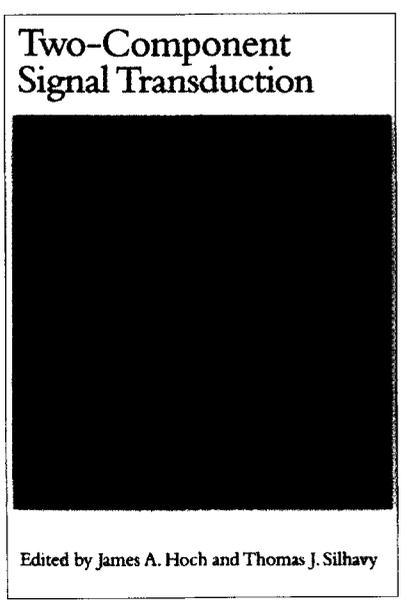
The publication of two key discoveries in 1986 resulted in a qualitative leap forward in our understanding of how bacteria process and act on information they gather about changing conditions in their environment. First, the database of amino acid sequences reached sufficient size that scientists studying a variety of regulatory processes in bacteria realized that they were all working with related pairs of proteins, christened 'two-component regulatory systems'[1]. Second, Ninfa and Magasanik showed that transient phosphorylation is the mechanism of communication between proteins in one such case, that of nitrogen assimilation[2]. The explosion of research triggered by these nearly simultaneous observations continues to this day.

We now know that each component protein in the regulatory pair typically contains a unique domain that carries out functions specific to the particular system, and a domain with conserved amino acid sequences that both define membership in a particular protein family and catalyze characteristic phosphoryl-group-transfer reactions. Histidine protein kinases are typically composed of an input domain that senses environmental conditions and a transmitter domain that autophosphorylates on a histidine residue in an input-sensitive manner. Response regulators are typically composed of a receiver domain that transfers the phosphoryl group from the histidine protein kinase to an aspartate residue of its own, and an output domain the function of which (usually transcriptional activation) is regulated by the phosphorylation state of the receiver. The basic mechanisms involved are sufficiently well conserved that initial generalizations based on one case have frequently been correct. Two-component regulatory systems appear to be ubiquitous among bacteria (although *Mycoplasma genitalium*, which has the smallest genome of any known free-living organism, lacks them[3]), and have also been found in one species of archaebacteria and at least four species of eukaryotes.



Two-component Signal Transduction is the first book to be published on this subject and therefore represents a significant milestone for this field of scientific inquiry. The book admirably fulfills a genuine need, as it is no longer feasible even for researchers studying two-component regulatory systems to read all the primary literature on this topic. Furthermore, the field is sufficiently mature and of broad enough interest that a reference work summarizing what is known in more detail than can be conveyed in a single review article should be useful to a variety of readers outside the field. The only remotely comparable compilation previously available is a much shorter and narrower collection of 17 review articles published last year in a special issue of the journal *Research in Microbiology*[4].

The book consists of 29 chapters grouped into six sections. The opening section covers General Principles about two-component regulatory systems. These chapters provide an excellent introduction to the subject, although an interesting opportunity was perhaps missed. Now that so much is known about two-component regulatory systems, it might have been useful to explore what general lessons about signal transduction mechanisms could be deduced by combining results from