# Common mechanism of secretory diarrhoea

**Nirmal Kumar Ganguly and Tanvir Kaur**

Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India

**The studies of intestinal electrolyte processes have eluded the attempts to identify the common mechanism involved in secretory type of diarrhoea. The pathogenesis of secretory diarrhoea involves the coupled interactions of $Ca^{2+}$, PI, PKC, PGs, intramural nerves and cholinergic muscarinic receptor activation. These interactions may result in increase in intracellular $Ca^{2+}$ levels. The increased $Ca^{2+}$ may thus serve an important intracellular mediator involved in the stimulation of intestinal fluid secretion.**

THE worldwide impact of acute infectious diarrhoeal diseases is immense, with an estimated 3–5 billion cases per year, resulting in 3–5 million deaths each year[1]. A survey reported more than 3 million deaths each year due to diarrhoea[2]. Eighty per cent of these deaths occur in children below two years of age[3]. It is estimated that the children less than two years of age in developing countries suffer 6–10 episodes of diarrhoea per year, thus approximately 17% of their life in this age group is spent with diarrhoea[4]. A close scrutiny of facts and figures available from National and International sources indicates that first two years of life of a child born in India continues to be crucial from health point of view. It has been estimated that approximately 3 million children die before completion of one year and one million die before attaining childhood every year and these deaths in one way or other are related to diarrhoeal diseases and their effects[5].

Infectious diarrhoea is generally secretory in nature and can result from bacterial, viral and parasitic infections. The bacteria commonly implicated in the etiology of diarrhoea include conventional enteropathogens like Shigella, Salmonella, enteropathogenic *E. coli* (EPEC), *Vibrio cholerae* and new enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), *Yersinia*, *Aeromonas*, campylobacter and recently isolated Vibrio 0139 synonym Bengal[6–9].

The understanding of underlying pathophysiological mechanisms of acute secretory diarrhoea is growing steadily, now thanks to the intense research interest in the physiology of intestinal electrolyte transport. Considerable insights have been obtained with respect to normal cellular mechanisms of ion transport, though the studies of intestinal electrolyte transport are complicated by several factors (Table 1).

## Structural basis for intestinal electrolyte transport

The epithelium of small intestine is located at the interfacial surface, the intestinal lumen is in continuity with external environment. The material absorbed from lumen must first traverse this epithelium to gain access to mucosal blood and lymph vessels.

## Structural organization of mucosa of small intestine

The luminal surface of small intestine is so organized that surface area is greatly amplified. Numerous microscopic mucosal villi increase the absorptive surface some seven to 14-fold[10]. Depending on the mammalian species and portion of small intestine, villus height and shape vary. Diseases that affect the mucosal function often perturb normal villus structure, thus affecting absorptive surface of small intestine[11].

The intestinal mucosa is divided into three distinct layers (Figure 1). The deepest is the muscularis mucosa that separates mucosa from submucosa. Its contractile properties help in the movement of villi and emptying of crypt luminal contents[12]. The lamina propria is the middle mucosal layer, which is bounded above by epithelium and below by muscularis mucosa. The lamina contains eosinophils, mast cells, fibroblasts, unmyelinated nerve fibres, blood and lymph vessels. The lamina propria provides support to intestinal epithelial cells and also contains blood vessels that nourish the epithelium. The third layer of intestinal mucosa constitutes a continuous sheet of epithelial cells which line the villi and crypts. The crypt epithelium is composed of undifferentiated cells, mucus secreting goblet cells, endocrine

**Table 1.** Regulation of cellular mechanisms of ion transport

1. Presence and simultaneous function of both absorptive and secretory processes.
2. Transmucosal movement of ions through both cellular and transcellular pathways.
3. Presence of multiple cell types.
4. Structural heterogeneity of epithelial cells.

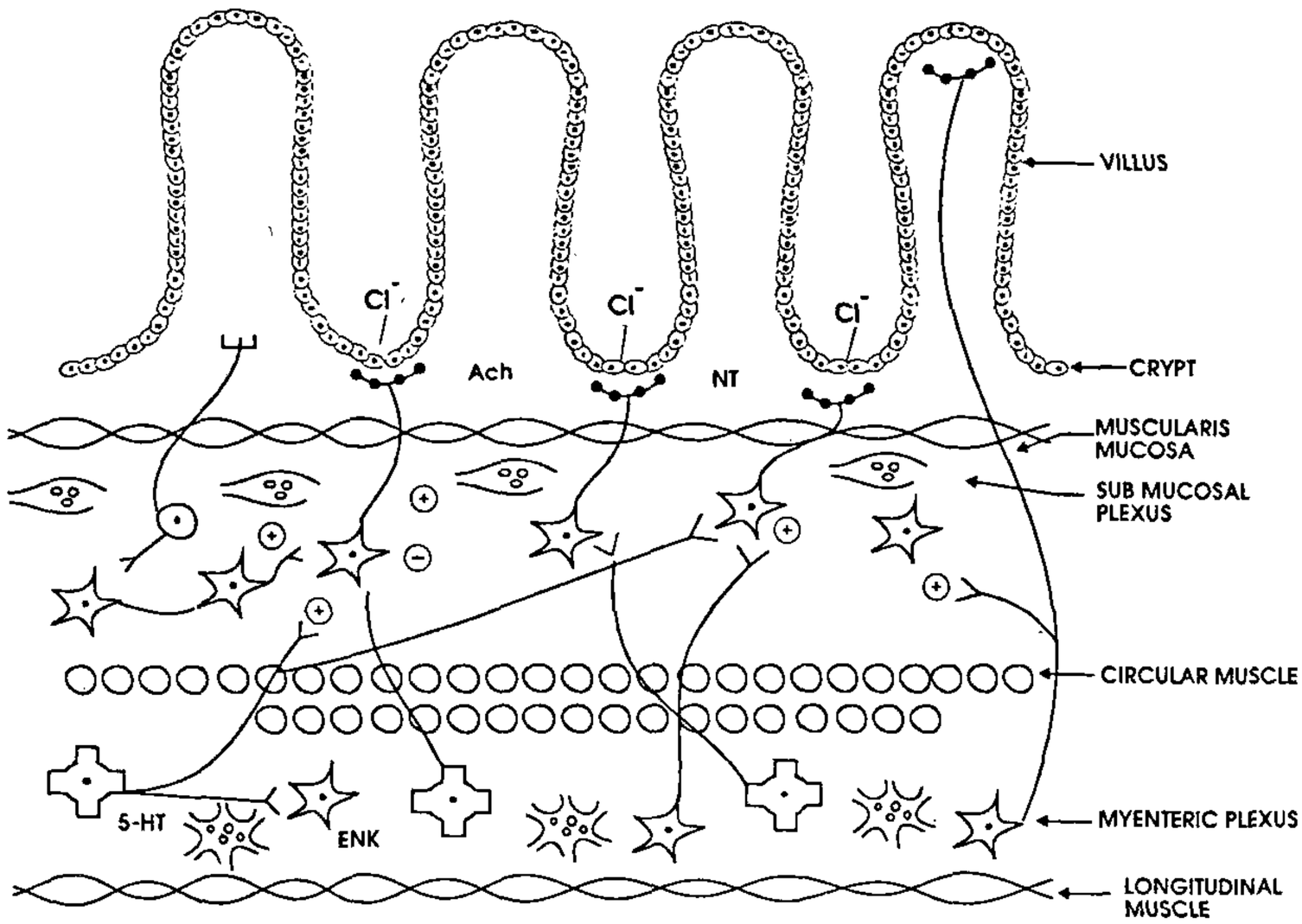


**Figure 1.** Functional morphology of mucosa of small intestine.

epithelial cells, tuft cells and Paneth cells with secretory granules. The epithelium lining the villi contains absorptive cells, termed as enterocytes, mucus secreting goblet cells, few endocrine epithelial cells, rare caveolated cells, cup cells. Additionally, a specialized epithelial cell, the M cell overlies the apex of Peyer's patches.

The functions of crypt epithelium include epithelial cell renewal, secretion into crypt lumen, electrolyte and water secretion and endocrine secretion both into the lamina propria and into the lumen. The major known function of villus epithelium is absorption of nutrients[12].

## Cellular mechanisms of ion transport

$Na^+$ and $Cl^-$ together are by far the major ions of fluid transported during absorption or secretion by small intestine. $Na^+$, in particular, plays a central role in the energetics of the intestinal absorption via the transcellular route. The fluid and electrolyte transported depend upon the net effect of absorptive and secretory processes.

$Na^+$ absorption mainly occurs by three mechanisms (Table 2). The driving force for $Na^+$ entry is the hydrolysis of ATP catalysed by $Na^+$, $K^+$-ATPase located at basolateral membrane of enterocytes[13]. This pump exchanges $Na^+$ out of $K^+$ in, thus establishing a steep electrochemical gradient for $Na^+$ entry from lumen (Figure 2). The small intestine of most mammals also possesses a secretory mechanism[14], which normally functions at a low basal level (Table 2). Previous findings support a distribution of secretory function to the crypt cells[15,16]. Recently, it has been demonstrated that crypts also absorb fluid and $Cl^-$ secretion occurs in presence of intracellular or extracellular messengers[17].

**Table 2.** Ion transport processes

| | |
|---|---|
| I. | *Absorptive processes* |
| | 1. Electrogenic sodium absorption |
| | 2. Non-electrolyte stimulated $Na^+$ absorption |
| | 3. Electroneutral sodium chloride absorption |
| II. | *Secretory processes* |
| | 1. Sodium secretion |
| | 2. Chloride secretion |

The mechanisms of electrolyte transport differ in various segments of mammalian intestine. In jejunum, $Na^+$ entry occurs by (i) $Na^+/H^+$ exchange, (ii) $Na^+$ substrate cotransport, (iii) $Na^+$–$PO_4^-$ and $Na^+$–$SO_4^{-2}$ co-transport but active $Cl^-$ absorption does not occur, there is no evidence of direct linking of $Na^+$ and $Cl^-$ transport, and no $Cl^-/HCO_3^-$ or $Cl^-/OH^-$ exchanger[18]. In mammalian ileum, $Na^+$ and $Cl^-$ transport are linked and Na-substrate mechanism is less prominent[19]. The mammalian distal colon demonstrates amiloride sensitive $Na^+$ absorption pathway whereas proximal colon possess a coupled NaCl transport mechanism[20].

## Pathophysiological mechanisms in diarrhoeal diseases

### *Cyclic nucleotides*

Cyclic nucleotides stimulate intestinal secretion by three potential mechanisms: (i) altered activity of ion trans-

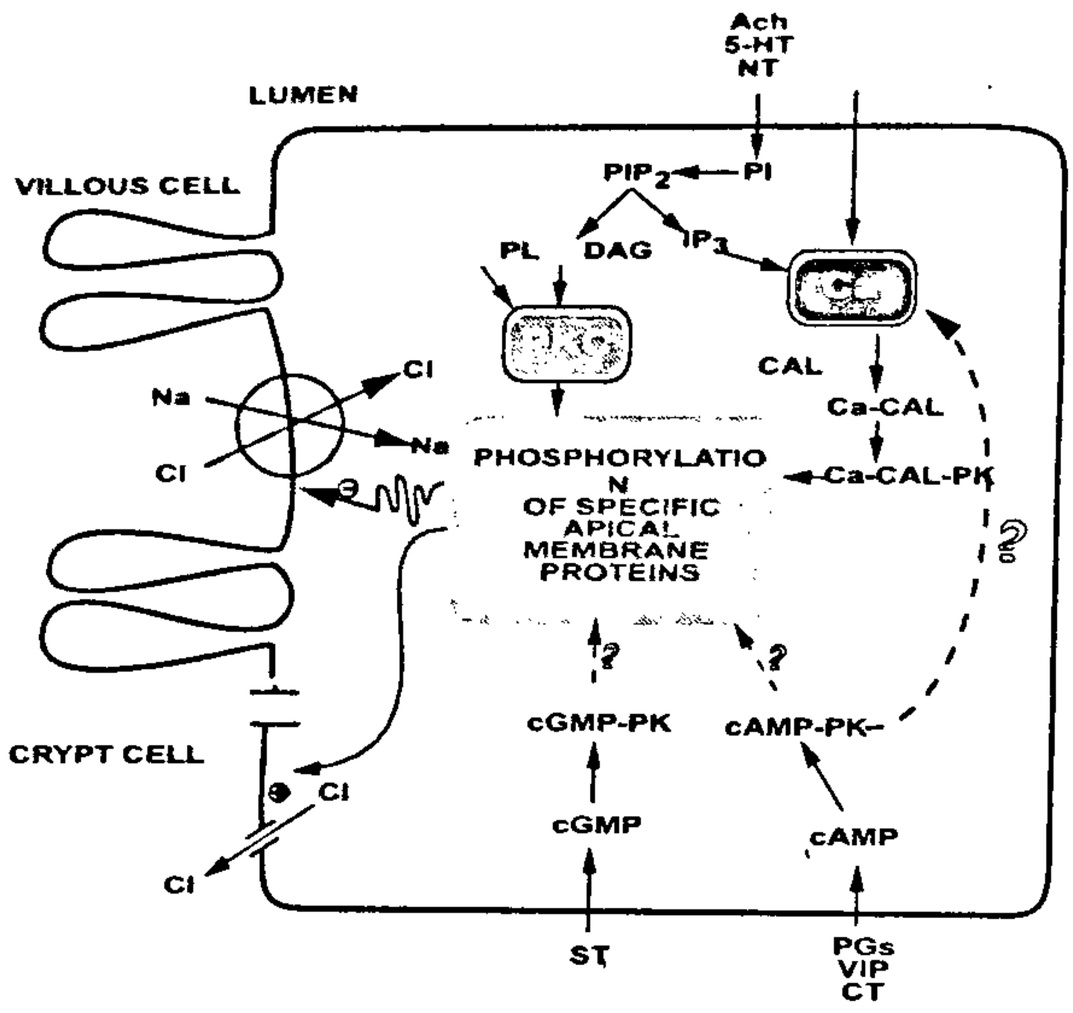


**Figure 2.** Regulation of intestinal secretion by extracellular mediators and intracellular messengers.

porters, (ii) modulation of tight junctions, and (iii) increase in intracellular calcium levels.

The elevation of cAMP or cGMP levels in intestinal epithelial cells stimulates active chloride secretion by inhibiting electroneutral NaCl absorption in intestinal epithelium[21]. Cyclic AMP acts via stimulation of A kinase with direct phosphorylation and activation of the major chloride channel identified in intestinal epithelial cells, the cystic fibrosis transmembrane conductance regulator (CFTR)[22]. Second, cyclic AMP regulates intestinal epithelial cell secretion via effects on cytoskeletal proteins. The chloride secretory response to cAMP is dependent on microtubules[23]. Further, the apical membrane recruitment of CFTR in T84 cells in response to cyclic AMP is dependent on microtubules but not calcium or F (filamentous) actin[24]. The mechanism by which activation of cAMP reorganizes F actin is not known. Besides its effect on ion transporters, the study on CT-treated animal tissues, *Campylobacter jejuni* and *Salmonella typhimurium* heat labile toxin suggest that cyclic AMP potentially contributes to intestinal secretion[25–27].

Lastly, cyclic AMP-dependent agonists stimulate increases in intracellular calcium $(Ca^{2+})_i$ in T84 intestinal epithelial cells. The cAMP-mediated increase in $(Ca^{2+})_i$ occurs via a pathway (Figure 2) distinct from that mediated by inositol triphosphate ($IP_3$) or protein kinase C (PKC)[28,29].

## Intracellular calcium

There are several potential mechanisms by which elevation in $(Ca^{2+})_i$ levels stimulates a net intestinal secretion. The elevation of $(Ca^{2+})_i$ levels alters the regulation of several ion-transporting proteins. The increased $(Ca^{2+})_i$ may activate calmodulin-dependent protein kinases (Ca–CaM) or calcium-dependent protein kinase (PKC)[30]. These activated kinases may phosphorylate membrane proteins that affect the activity of apical membrane $Na^+/H^+$, exchanger (Figure 2) resulting the decreased $Na^+$ absorption[31]. Secondly, Ca–CaM dependent protein kinases and PKC may activate CFTR[32]. Undoubtedly, elevated calcium is enough signal to stimulate chloride secretion in intestinal epithelial cells in the absence of additional second messengers. The previous studies based on evaluating the role of calcium in the presence of its agonists and antagonists suggest its involvement in pathogenesis of many diarrhoeagenic organisms[26,33–36]. Additionally, calcium has been known to regulate tight junctions, suggesting that changes in $(Ca^{2+})_i$ levels modulate the intestinal permeability and contribute to intestinal secretion[37].

## Protein kinase C

The PKC family consists of multiple isoenzymes that differ from each other in one way or other[38]. Specific isoforms of PKC are present in various intestinal cell types. The metabolism of phosphatidyl inositol by phospholipase C (PLC) results in generation of $IP_3$ and diacylglycerol (DAG). $IP_3$ releases calcium from intracellular calcium stores whereas DAG activates PKC. The role of PKC activation in stimulating intestinal secretion has been examined by use of activators and inhibitors of PKC with help of Ussing chambers[26,35,39]. Besides causing intestinal secretion, PKC activation also leads to dissembly of intestinal epithelial cell cytoskeleton and modulation of tight junction[40]. Thus it suggests that PKC activation may contribute to intestinal secretion through direct effects on ion transporters as well as through regulation of paracellular transport pathway.

## Phospholipases

The activation of phospholipases leads to changes in intracellular mediators which regulate transport of fluid and electrolytes. The most studied examples are (i) release of arachidonic acid metabolites by the activation of $PLA_2$ and (ii) release of intracellular calcium through activation of PLC. Activation of $PLA_2$ leads to the release of AA metabolites, viz. prostaglandins (cyclooxygenase pathway products) and leukotrienes (lipooxygenase pathway products). Certain prostaglandins and leukotrienes may serve to amplify the secretory response for example, prostaglandin $I_2$ ($PGI_2$) can activate enteric nervous system and leukotriene B4 stimulates the

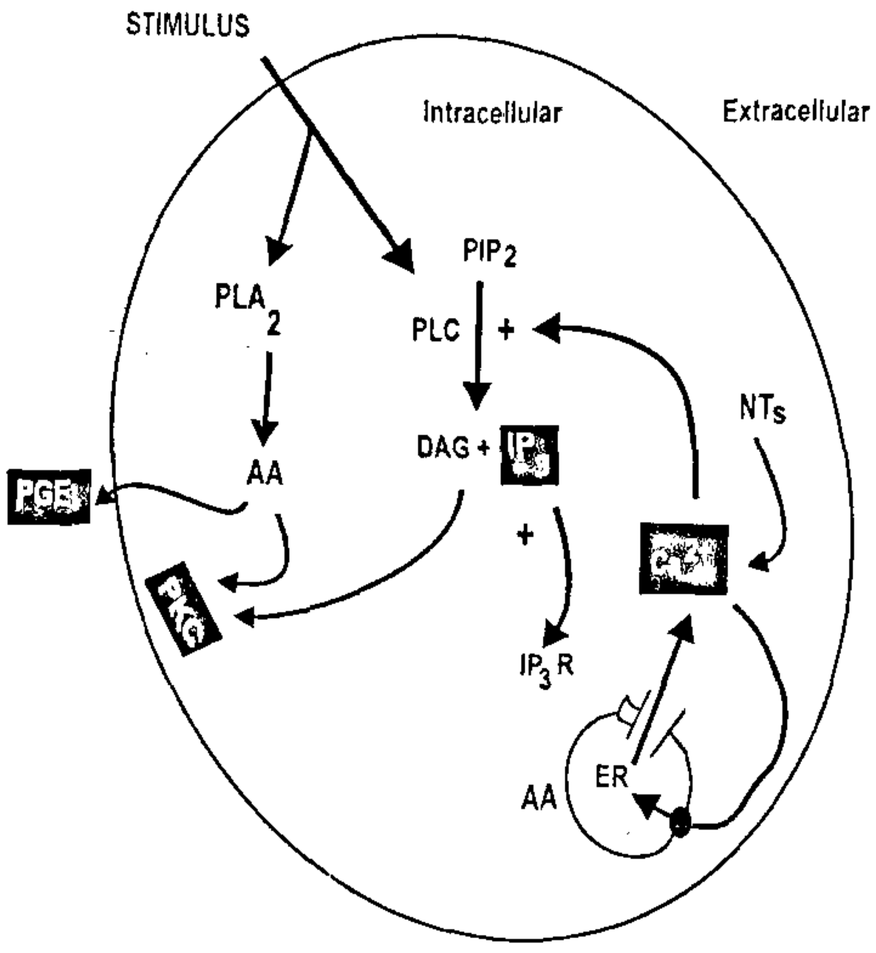


**Figure 3.** Proposed pathophysiological mechanism(s) involved in secretory diarrhoea.

recruitment of PMNs, leading to an inflammatory response in intestine. Activation of PKC regulates ion transport through the release of calcium and PKC activators as summarized above[41]. Recently, additional signalling pathways that involve the activation of PLD and phosphatidyl specific PLC have been identified[38,42]. These pathways lead to the release of lipid metabolites (e.g. diacyl glycerol and phosphatidic acid) that regulate specific cell functions.

## Enteric nervous system

Considerable evidence exists regarding the involvement of one or more components of enteric nervous system in secretory responses to bacterial toxins[43]. Neural pathways appear to involve villus to crypt neural reflexes. The neuropeptides (Figure 1) such as those secreting substance P, 5-HT, vasoactive intestinal peptide (VIP)[44,45] and acetylcholine have been implicated in the secretory responses to *C. difficile* toxin A, CT, *E. coli* heat stable enterotoxins (STa and STb) and Shigella toxin[36,46–48]. Several bacterial pathogens and/or their toxins have been shown to alter intestinal myoelectric patterns. These include CT, *E. coli* STa, wild type and recombinant *V. cholerae* strains, Shigella toxin and various classes of *E. coli* [e.g. enteropathogenic *E. coli* (EPEC), ETEC and enteroinvasive *E. coli* (EIEC)][36,49–51].

## Summary

Diarrhoea is one of the major causes of mortality during infancy and early childhood in developing countries. The underlying pathophysiological mechanisms in secretory diarrhoea involves the complex interaction of several biochemical pathways. Calcium may be located at the cross over point of various pathways involving PKC, prostaglandins, intramural nerves and cyclic nucleotides. Increased calcium (either through extracellular calcium or release of calcium from intracellular stores) may activate PKC by its translocation from cytosolic to membrane, thereby phosphorylate membrane proteins. The sequence of interaction leading to an increase in intracellular $Ca^{2+}$ appears central to the control of diarrhoea modulating ion transport through activation of protein kinase C (Figure 3).

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# Molecular biology and biotechnology of higher plant nitrate reductases

**H. S. Srivastava and Niharika Shankar**

Department of Plant Science, Rohilkhand University, Bareilly 243 006, India

**Nitrate reductase (NR) catalysing the reduction of nitrate to nitrite is the first and rate limiting enzyme in the assimilation of nitrate by the plants. At least two of its isoforms; a NADH specific and a NAD(P)H bispecific have been characterized. In this article, the physico-chemical properties of NADH specific isoform, which is the principal isoform of nitrate assimilation, have been described. The properties of cloned genes and the production of transgenic plants with altered NR activity, and mechanism of induction of NR by nitrate and cytokinins, repression by glutamine and post-translational modification of NR protein through reversible phosphorylation by light–dark transitions have also been accounted for. The characterization of NAD(P)H : NR gene and some regulatory aspects of this isoform have also been described. The article demonstrates that it is possible to produce mutants and transgenics with altered structure and function of NR, with an ultimate aim to affect qualitative and quantitative improvement of crop plants.**

NITRATE reductase (NR) catalysing the reduction of nitrate to nitrite is the first enzyme in the assimilation of nitrate by plants. The activity is considered to be the rate-limiting step in nitrate assimilation, which is often positively correlated with the total protein and nitrogen contents and sometimes also with the overall productivity of the plants[1]. Three isoforms of this enzyme have been described from soybean[2], viz. (i) A nitrate inducible NADH : NR (E.C.1.6.6.1) with a pH optimum of 7.5, (ii) A constitutive or inducible bispecific NAD(P)H : NR (E.C.1.6.6.2) with a optimum pH of 6.5, and (iii) A constitutive NADH : NR (E.C. number not yet assigned), with a pH optimum of 6.5. The inducible NADH : NR and the bispecific NAD(P)H : NR are usually found in close association with various plants and perhaps both are involved in nitrate assimilation. The NAD(P)H : NR however, is considered to be associated with some other functions also, such as with the transport of nitrate across the membranes and with the dissimilatory release of oxygen in anaerobic environment[3]. The evidences for these alternate functions are yet to be known, although the observation that NAD(P)H : NR isoform was localized principally on the plasma membrane[4] is a strong indicator of its role in nitrate acquisition and transport. NR has been used as a biotechnological tool/product also. Mellor