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Calcium and 1,25(OH)$_2$D: interacting drivers of epidermal differentiation☆

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Abstract

Both calcium and 1,25(OH)$_2$D promote the differentiation of keratinocytes in vitro. The autocrine or paracrine production of 1,25(OH)$_2$D by keratinocytes combined with the critical role of the epidermal calcium gradient in regulating keratinocyte differentiation in vivo suggest the physiologic importance of this interaction. The interactions occur at a number of levels. Calcium and 1,25(OH)$_2$D synergistically induce involucrin, a protein critical for cornified envelope formation. The involucrin promoter contains an AP-1 site essential for calcium and 1,25(OH)$_2$D induction and an adjacent VDRE essential for 1,25(OH)$_2$D but not calcium induction. Calcium regulates coactivator complexes that bind to the Vitamin D receptor (VDR). Nuclear extracts from cells grown in low calcium contain an abundance of DRIP$_{205}$, whereas calcium induced differentiation leads to reduced DRIP$_{205}$ and increased SRC 3 which replaces DRIP in its binding to the VDR. In vivo models support the importance of 1,25(OH)$_2$D–calcium interactions in epidermal differentiation. The epidermis of *1αOHase* null mice fails to form a normal calcium gradient, has reduced expression of proteins critical for barrier function, and shows little recovery of the permeability barrier when disrupted. Thus in vivo and in vitro, calcium and 1,25(OH)$_2$D interact at multiple levels to regulate epidermal differentiation.

Keywords: Calcium; 1,25(OH)$_2$D; Keratinocyte; Epidermis; Differentiation; Coactivators; Transcription; Involucrin; Transglutaminase

1. Calcium induced differentiation

The epidermis in vivo and keratinocytes from the epidermis in vitro provide excellent models for the study of differentiation by normal cells. A cartoon of the epidermis demonstrating the differentiation process in vivo as the cells leave the stratum basale and progress toward the stratum corneum is depicted in Fig. 1. Much attention has been paid to the critical role of calcium in this process [1–3]. If keratinocytes are grown at calcium concentrations below 0.07 mM, they continue to proliferate but either fail or are slow to develop intercellular contacts, stratify little if at all, and fail or are slow to form cornified envelopes. Acutely increasing the extracellular calcium concentration (Cao) above 0.1 mM (calcium switch) leads to the rapid redistribution of desmoplakin, cadherins, integrins, catenins, plakoglobulin, vinculin, and actinin from the cytosol to the membrane where they participate in the formation of intercellular contacts [1,4]. Calcium also stimulates the redistribution to the membrane of protein kinase Cα (PKCα) [5], the tyrosine-phosphorylated p62 associated protein of ras GAP [6] and calmodulin where they further the calcium signaling process. Within hours of the calcium switch keratinocytes change from making the basal keratins K5 and K14 and begin making keratins K1 and K10 [3] followed, subsequently, by increased levels of profilaggrin (the precursor of filaggrin, an intermediate filament associated protein), involucrin and loricrin (precursors for the cornified envelope) [7,8] and transglutaminase [9,10]. Loricrin, involucrin and other proteins are cross linked into the insoluble cornified envelope by the calcium sensitive, membrane bound form of transglutaminase [9]. Within 1–2 days after the calcium switch cornified envelope formation is apparent [2,10], paralleling transglutaminase activation. The induction of these proteins represents a genomic action (likely indirect) of calcium as indicated by a calcium-induced increase in mRNA levels and transcription rates [3,8,11,12]. The relevance of calcium-induced differentiation in vitro to the in vivo situation is indicated by the steep gradient of calcium within the epidermis, with the highest levels in the uppermost (most differentiated) nucleated layers [13] (Fig. 2). The calcium gradient helps maintain the differentiated function of the epidermis, including the permeability barrier. Disruption of this barrier leads to

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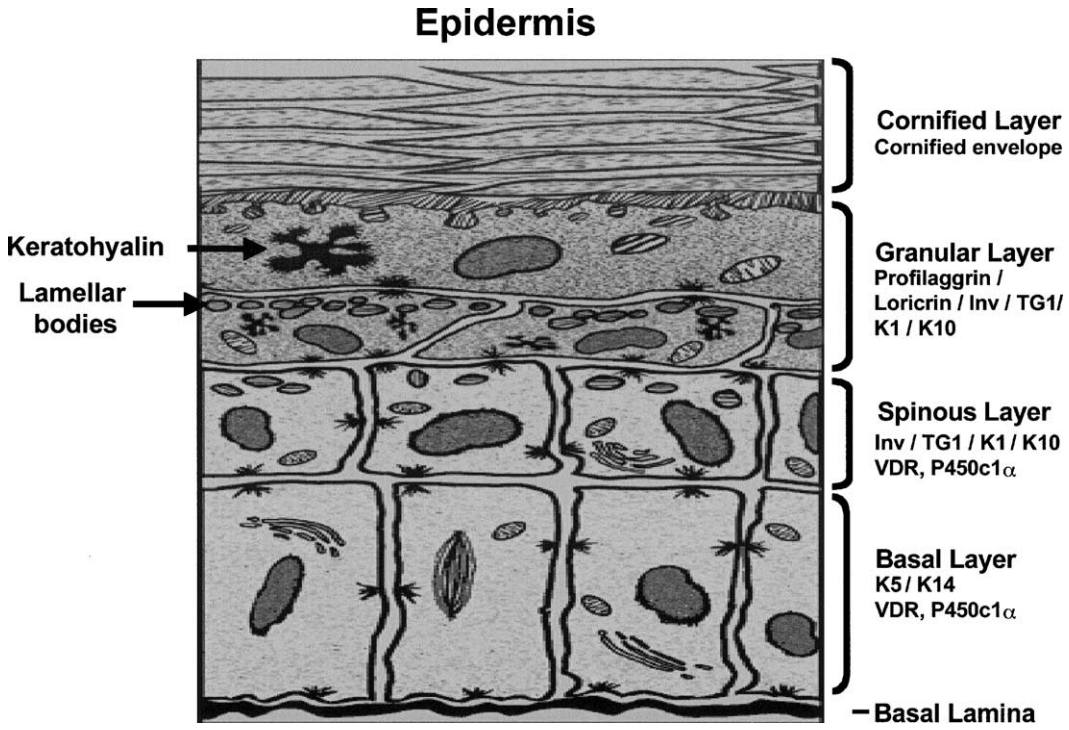

Fig. 1. A cartoon of the epidermis illustrating the different strata, and the sequential expression of proteins that mark the differentiation of the keratinocyte as it leaves the stratum basale and ascends to the stratum corneum from which it is eventually sloughed. The VDR and 1αOHase are predominately in the basal layers. The early markers of differentiation, keratins 1 and 10, appear in the spinous layer along with involucrin, one of the substrates for the cornified envelope, and transglutaminase, which crosslinks involucrin and other substrates into the cornified envelope. Loricrin, a major component of the cornified envelope, and profilaggrin, a bundling protein for the keratin filaments, appear in the stratum granulosum in the characteristic granules that give this layer its name. The contents of lamellar bodies, containing lipids and lipid processing enzymes, the protein containing granules, and the keratin bundles combine to form the enucleate stratum corneum, providing the barrier function for the skin.

loss of the calcium gradient followed by increased proliferation and decreased expression of various differentiation markers (loricrin, profilaggrin, and involucrin) [14].

2. Calcium signaling in the keratinocyte

The response of the keratinocyte to calcium is complex (Fig. 3). The calcium switch results in a multiphasic increase in intracellular free calcium concentration (Cai)—a prolonged plateau follows the initial spike of increased Cai [15]. Agents like ATP and EGF that only stimulate the transient increase in Cai are unable to stimulate keratinocyte differentiation [16]. Key components of the calcium signaling process are as follows.

2.1. The calcium receptor

The response of the keratinocyte to calcium resembles that of the parathyroid cell. The parathyroid cell senses Cao via a seven transmembrane domain, G protein coupled receptor (CaR) [17], and we have identified the same receptor in the keratinocyte [18,19]. However, we also observed that the keratinocyte produces an alternatively spliced variant of the CaR (CaRalt) as it differentiates, which lacks exon 5 [19]. The currently available mouse model in which the full length CaR (CaRfl) was knocked out by insertion of a neomycin cassette into exon 5 continues to produce CaRalt [20]. Nevertheless, the epidermis of this mouse is abnormal, contains markedly lower levels of the terminal differentiation markers loricrin and profilaggrin, and the keratinocytes from this mouse fail to respond to calcium with a substantial rise in Cai [20]. This suggests that the full length CaR is required for terminal differentiation of mouse epidermis. Blocking the production of the CaR (both forms) with an antisense construct in keratinocytes decreased the ability of calcium to raise Cai and induce the INV and *TG* genes [21].

2.2. Calcium channels

Several calcium channels have been identified in the keratinocyte membrane, and these are all candidates for mediating calcium induced calcium influx [22–24]. Of particular interest to our laboratory are trp channels which function like store-activated channels (SOC) by responding to activation of inositol tris phosphate receptors (IP_3R) and depletion of intracellular stores with increased calcium influx. We have identified a number of these channels in keratinocytes. Inhibitors of IP_3R and phospholipase C (PLC) activity block their function, whereas inhibition of CaR expression reduces

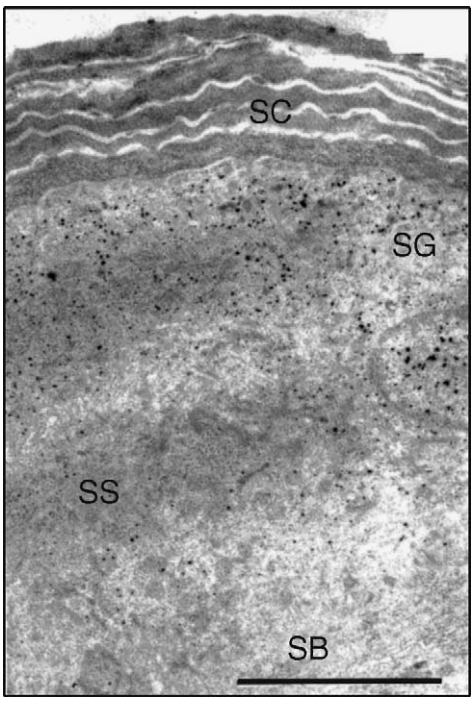

Fig. 2. The calcium gradient in the epidermis as demonstrated by ion capture cytochemistry. The black dots indicate calcium complexed to pyroantimoniate. Very little calcium is found in the stratum basale (SB). As one ascends through the stratum spinosum (SS) increasing amounts of calcium are found, reaching a peak in the stratum granulosum (SG). Very little calcium is found in the stratum corneum (SC). (Picture courtesy of Dr. Peter Elias.)

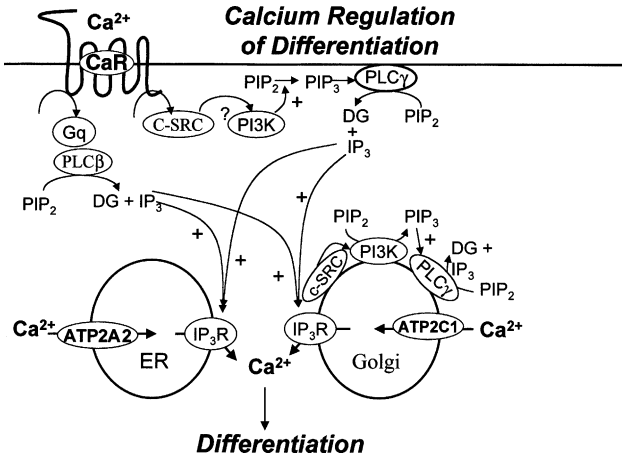

Fig. 3. A model of the calcium signaling events in the keratinocyte. Calcium activates its signaling pathways at least in part via its receptor (CaR). The CaR stimulates the hydrolysis of phosphatidyl inositol bis phosphate (PIP_2) by activating phospholipase C-β. This releases two second messengers, inositol tris phosphate (IP_3) and diacyl glycerol (DG). IP_3 in turn activates its receptor IP_3R to release calcium from intracellular stores. DG activates protein kinases, not shown here, but which are also important for calcium signaling. Calcium, presumably through the CaR, also activates PLC-γ by a mechanism mediated by src kinases and phosphatidyl inositol 3 kinases (PI3K). Both Golgi and ER participate in regulating intracellular calcium. Each has its own calcium pump, and each has the IP_3R. An interacting complex of calcium signaling proteins including CaR is found in the Golgi, suggesting it may be the more important subcellular organelle with respect to calcium signaling. Calcium channels, including store operated channels, are found in the keratinocyte and play important roles in calcium influx, but they have not been included in this model.

intracellular calcium stores and promotes SOC activity [25]. Our finding of CaR, IP_3R, the Golgi calcium pump ATP2C1 (called PMR1 in yeast), and PLC-γ1 in the same intracellular complex suggests an interaction among these molecules that regulates both calcium release from intracellular stores and calcium influx through SOC and possibly other plasma membrane calcium channels [26].

2.3. Phospholipase C

Inositol 1,4,5-tris-phosphate (IP_3) and diacylglycerol (DG) levels increase within seconds to minutes after the calcium switch implicating activation of the phospholipase C (PLC) pathway [27] and leading to the rise in Cai. Similar to Cai, the levels of inositol phosphates (IPs) remain elevated for hours after the calcium switch, indicating ongoing activation of the PLC pathway. This prolonged increase in IPs appears to be due to calcium activation of PLC-γ1 [28], although the initial increase in IP_3 and Cai after the calcium switch is probably mediated by PLC-β (Xie and Bikle, unpublished). This extended activation of PLC-γ1 is accompanied by a calcium induced increase in src family tyrosine kinases that are required for calcium activation of PLC-γ1 [29,30]. Blocking PLC-γ1 production using an antisense construct blocks calcium induced differentiation [31].

3. 1,25(OH)$_2$D stimulated differentiation

1,25(OH)$_2$D promotes keratinocyte differentiation via many of the same pathways as calcium [32], although in the absence of calcium 1,25(OH)$_2$D stimulated differentiation is not as complete. 1,25(OH)$_2$D is likely to be an autocrine or paracrine factor for epidermal differentiation since it is produced by the keratinocyte, but under normal circumstances keratinocyte production of 1,25(OH)$_2$D does not appear to contribute to circulating levels [33,34]. The receptors for and the production of 1,25(OH)$_2$D vary with differentiation [35] in a manner that suggests feedback regulation; both are reduced in the later stages of differentiation. 1,25(OH)$_2$D increases the expression of involucrin and transglutaminase [11,12] leading to increased cornified envelope formation [36–39]. These effects of 1,25(OH)$_2$D can be reproduced by 25OHD [40,41], presumably because of endogenous conversion of 25OHD to 1,25(OH)$_2$D, but are not observed with the biologically inactive β isomer of 1,25(OH)$_2$D[37].

4. Cellular mechanisms for 1,25(OH)$_2$D actions

The 1,25(OH)$_2$D receptor (VDR) is critical for the genomic actions of 1,25(OH)$_2$D; its role, if any, in potential

nongenomic actions of $1,25(OH)_2D$ remains to be determined. An acute increase in Cai associated with increased phosphoinositide turnover, increased IP_3 and DG, and redistribution of protein kinase C (PKC) to the membrane have been observed in several studies [42–44]. Down-regulation of PKC and inhibition of its activity have been reported to block the ability of $1,25(OH)_2D$ to stimulate cornified envelope formation [43]. However, the role of PKC in mediating or interacting with $1,25(OH)_2D$ in its effects on keratinocyte differentiation remains virtually unexplored.

5. Calcium–$1,25(OH)_2D$ interactions

Fig. 4 illustrates points at which $1,25(OH)_2D$ interfaces with the calcium signaling system. The expression of the CaR is increased by $1,25(OH)_2D$, making the keratinocyte more sensitive to the prodifferentiating actions of calcium [45]. All of the PLC family members are induced by $1,25(OH)_2D$ [46] as they are by calcium [31], and blocking PLC-γ1 expression prevents both $1,25(OH)_2D$ and calcium stimulated differentiation [31,47]. The VDRE in the PLC-γ1 promoter has been identified (a DR6) [48], but not the calcium response element. Calcium and $1,25(OH)_2D$ also interact in their ability to induce involucrin and transglutaminase [12]. At least one explanation for the synergism in the induction of involucrin is that the calcium response element (CaRE) and Vitamin D response element (VDRE) in the involucrin promoter are quite close spatially [48,49]. Mutations in the AP-1 site within the CaRE block both calcium and $1,25(OH)_2D$ induction of the involucrin gene, but mutations of the VDRE block only its response to $1,25(OH)_2D$. An additional explanation lies in the observation that calcium, at least indirectly, regulates coactivator complexes that bind to the Vitamin D receptor (VDR). Nuclear extracts from cells grown in low calcium contain an abundance of $DRIP_{205}$, whereas calcium induced differentiation leads to reduced $DRIP_{205}$ and increased SRC 3 which replaces DRIP in its binding to the VDR. Different genes respond differently to the different VDR–coactivator complexes (Oda and Bikle, elsewhere in this symposium).

6. In vivo studies

The recent availability of mice with mutated *VDR* and *1α-hydroxylase* (*1αOHase*) genes has expanded our understanding of the role of $1,25(OH)_2D$ in epidermal differentiation. Although the most striking feature of the *VDR* null mouse is the development of alopecia (also found in many patients with mutations in *VDR*), these mice also exhibit a defect in epidermal differentiation as shown by reduced levels of involucrin, profilaggrin, and loricrin and loss of keratohyalin granules [50]. However, these changes can be reversed when the animals are placed on a high calcium diet, unlike the abnormalities in the hair follicle (Bikle et al. unpublished). A different phenotype is observed in the *1αOHase* null mouse. These mice also show a reduction in levels of the epidermal differentiation markers, but unlike the VDR null animals, a high calcium diet does not rescue this phenotype. Furthermore, the *1αOHase* null animals fail to develop a normal calcium gradient in their epidermis, and when their permeability barrier is disrupted have a retarded recovery of barrier function associated with

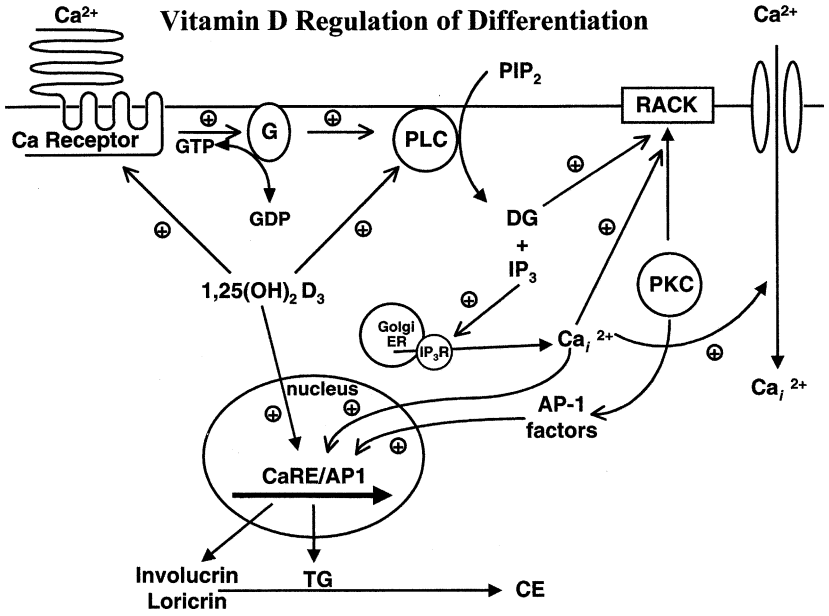

Fig. 4. The modulatory role of $1,25(OH)_2D$ in calcium signaling. $1,25(OH)_2D$ increases the expression of CaR and the PLC family members. This increases the sensitivity of the keratinocyte to calcium activation. $1,25(OH)_2D$ also induces a number of proteins required for differentiation such as involucrin and transglutaminase (TG) directly involved in cornified envelope (CE) formation.

an impaired reestablishment of the calcium gradient [51]. Thus, these in vivo studies emphasize the significance of the $1,25(OH)_2D$–calcium interactions demonstrated in vivo in understanding keratinocyte differentiation in vivo.

7. Summary and conclusions

Calcium appears to be the major regulator of keratinocyte differentiation. $1,25(OH)_2D$, however, plays an important modulatory role in that it induces a number of key elements in the calcium signaling pathway including the CaR and the PLCs required for calcium responsiveness. Furthermore, $1,25(OH)_2D$ can induce a number of proteins directly involved in differentiation such as involucrin and transglutaminase. The keratinocyte clearly has evolved compensatory mechanisms in that many of the proteins induced by $1,25(OH)_2D$ can also be induced by calcium in the absence of $1,25(OH)_2D$. Nevertheless, our recent findings with the *1αOHase* null mouse indicate that $1,25(OH)_2D$, most likely produced locally, is required for maintenance of the calcium gradient in vivo, and that maintenance of this gradient is required for normal epidermal differentiation and function.

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References

[1] H. Hennings, D. Michael, C. Cheng, P. Steinert, K. Holbrook, S.H. Yuspa, Calcium regulation of growth and differentiation of mouse epidermal cells in culture, Cell 19 (1980) 245.
[2] S. Pillai, D.D. Bikle, M.L. Mancianti, P. Cline, M. Hincenbergs, Calcium regulation of growth and differentiation of normal human keratinocytes: modulation of differentiation competence by stages of growth and extracellular calcium, J. Cell Physiol. 143 (1990) 294.
[3] S.H. Yuspa, A.E. Kilkenny, P.M. Steinert, D.R. Roop, Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in vitro, J. Cell Biol. 109 (1989) 1207.
[4] V.M. Braga, K.J. Hodivala, F.M. Watt, Calcium-induced changes in distribution and solubility of cadherins, integrins and their associated cytoplasmic proteins in human keratinocytes, Cell Adhes. Commun. 3 (1995) 201.
[5] M.F. Denning, A.A. Dlugosz, E.K. Williams, Z. Szallasi, P.M. Blumberg, S.H. Yuspa, Specific protein kinase C isozymes mediate the induction of keratinocyte differentiation markers by calcium, Cell Growth Differ. 6 (1995) 149.
[6] E. Filvaroff, E. Calautti, M. Reiss, G.P. Dotto, Functional evidence for an extracellular calcium receptor mechanism triggering tyrosine kinase activation associated with mouse keratinocyte differentiation, J. Biol. Chem. 269 (1994) 21735.
[7] R.H. Rice, H. Green, Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: activation of the cross-linking by calcium ions, Cell 18 (1979) 681.
[8] D. Hohl, U. Lichti, D. Breitkreutz, P.M. Steinert, D.R. Roop, Transcription of the human loricrin gene in vitro is induced by calcium and cell density and suppressed by retinoic acid, J. Invest. Dermatol. 96 (1991) 414.
[9] S.M. Thacher, R.H. Rice, Keratinocyte-specific transglutaminase of cultured human epidermal cells: relation to cross-linked envelope formation and terminal differentiation, Cell 40 (1985) 685.
[10] H. Hennings, P. Steinert, M.M. Buxman, Calcium induction of transglutaminase and the formation of epsilon (gamma-glutamyl) lysine cross-links in cultured mouse epidermal cells, Biochem. Biophys. Res. Commun. 102 (1981) 739.
[11] D.F. Gibson, A.V. Ratnam, D.D. Bikle, Evidence for separate control mechanisms at the message, protein, and enzyme activation levels for transglutaminase during calcium-induced differentiation of normal and transformed human keratinocytes, J. Invest. Dermatol. 106 (1996) 154.
[12] M.J. Su, D.D. Bikle, M.L. Mancianti, S. Pillai, 1,25-Dihydroxy Vitamin D3 potentiates the keratinocyte response to calcium, J. Biol. Chem. 269 (1994) 14723.
[13] G.K. Menon, S. Grayson, P.M. Elias, Ionic calcium reservoirs in mammalian epidermis: ultrastructural localization by ion-capture cytochemistry, J. Invest. Dermatol. 84 (1985) 508.
[14] P.M. Elias, S.K. Ahn, M. Denda, B.E. Brown, D. Crumrine, L.K. Kimutai, L. Komuves, S.H. Lee, K.R. Feingold, Modulations in epidermal calcium regulate the expression of differentiation-specific markers, J. Invest. Dermatol. 119 (2002) 1128.
[15] G.R. Sharpe, J.I. Gillespie, J.R. Greenwell, An increase in intracellular free calcium is an early event during differentiation of cultured human keratinocytes, FEBS Lett. 254 (1989) 25.
[16] S. Pillai, D.D. Bikle, Adenosine triphosphate stimulates phosphoinositide metabolism, mobilizes intracellular calcium, and inhibits terminal differentiation of human epidermal keratinocytes, J. Clin. Invest. 90 (1992) 42.
[17] E.M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M.A. Hediger, J. Lytton, S.C. Hebert, Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid, Nature 366 (1993) 575.
[18] D.D. Bikle, A. Ratnam, T. Mauro, J. Harris, S. Pillai, Changes in calcium responsiveness and handling during keratinocyte differentiation: potential role of the calcium receptor, J. Clin. Invest. 97 (1996) 1085.
[19] Y. Oda, C.L. Tu, S. Pillai, D.D. Bikle, The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation, J. Biol. Chem. 273 (1998) 23344.
[20] Y. Oda, C.L. Tu, W. Chang, D. Crumrine, L. Kömüves, T. Mauro, P.M. Elias, D.D. Bikle, The calcium sensing receptor and its alternatively spliced form in murine epidermal differentiation, J. Biol. Chem. 275 (2000) 1183.
[21] C.L. Tu, W. Chang, D.D. Bikle, The extracellular calcium-sensing receptor is required for calcium-induced differentiation in human keratinocytes, J. Biol. Chem. 276 (2001) 41079.
[22] T.M. Mauro, P.A. Pappone, R.R. Isseroff, Extracellular calcium affects the membrane currents of cultured human keratinocytes, J. Cell. Physiol. 143 (1990) 13.
[23] T.M. Mauro, R.R. Isseroff, R. Lasarow, P.A. Pappone, Ion channels are linked to differentiation in keratinocytes, J. Membr. Biol. 132 (1993) 201.
[24] S.A. Grando, R.M. Horton, T.M. Mauro, D.A. Kist, T.X. Lee, M.V. Dahl, Activation of keratinocyte nicotinic cholinergic receptors stimulates calcium influx and enhances cell differentiation, J. Invest. Dermatol. 107 (1996) 412.
[25] C.L. Tu, D.D. Bikle, Trp proteins mediate the store-operated Ca^{2+} influx in human epidermal keratinocytes, J. Invest. Dermatol. 119 (2002) 271.
[26] C.L. Tu, D.D. Bikle, Calcium sensing receptor forms a signaling complex with IP3R, PLC-gamma-1 and Golgi Ca-ATPase in keratinocytes, J. Invest. Dermatol. 121 (2003) 725.

[27] S. Jaken, S.H. Yuspa, Early signals for keratinocyte differentiation: role of Ca^{2+}-mediated inositol lipid metabolism in normal and neoplastic epidermal cells, Carcinogenesis 9 (1988) 1033.
[28] K. Punnonen, M. Denning, E. Lee, L. Li, S.G. Rhee, S.H. Yuspa, Keratinocyte differentiation is associated with changes in the expression and regulation of phospholipase C isoenzymes, J. Invest. Dermatol. 101 (1993) 719.
[29] Y. Zhao, M. Sudol, H. Hanafusa, J. Krueger, Increased tyrosine kinase activity of c-Src during calcium-induced keratinocyte differentiation, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 8298.
[30] E. Calautti, C. Missero, P.L. Stein, R.M. Ezzell, G.P. Dotto, fyn tyrosine kinase is involved in keratinocyte differentiation control, Genes Dev. 9 (1995) 2279.
[31] Z. Xie, D.D. Bikle, Phospholipase C-gamma-1 is required for calcium-induced keratinocyte differentiation, J. Biol. Chem. 274 (1999) 20421.
[32] D.D. Bikle, S. Pillai, Vitamin D, calcium, and epidermal differentiation, Endocr. Rev. 14 (1993) 3.
[33] D.D. Bikle, M.K. Nemanic, J.O. Whitney, P.W. Elias, Neonatal human foreskin keratinocytes produce 1,25-dihydroxyVitamin D3, Biochemistry 25 (1986) 1545.
[34] D.D. Bikle, M.K. Nemanic, E. Gee, P. Elias, 1,25-Dihydroxy Vitamin D3 production by human keratinocytes: kinetics and regulation, J. Clin. Invest. 78 (1986) 557.
[35] S. Pillai, D.D. Bikle, P.M. Elias, 1,25-Dihydroxy Vitamin D production and receptor binding in human keratinocytes varies with differentiation, J. Biol. Chem. 263 (1988) 5390.
[36] J. Hosomi, J. Hosoi, E. Abe, T. Suda, T. Kuroki, Regulation of terminal differentiation of cultured mouse epidermal cells by 1-alpha,25-dihydroxy Vitamin D3, Endocrinology 113 (1983) 1950.
[37] E.L. Smith, N.C. Walworth, M.F. Holick, Effect of 1-alpha,25-dihydroxy Vitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions, J. Invest. Dermatol. 86 (1986) 709.
[38] S. Pillai, D.D. Bikle, Role of intracellular-free calcium in the cornified envelope formation of keratinocytes: differences in the mode of action of extracellular calcium and 1,25-dihydroxy Vitamin D3, J. Cell Physiol. 146 (1991) 94.
[39] J.A. McLane, M. Katz, N. Abdelkader, Effect of 1,25-dihydroxy Vitamin D3 on human keratinocytes grown under different culture conditions, In Vitro Cell Dev. Biol. 26 (1990) 379.
[40] K. Matsumoto, Y. Azuma, M. Kiyoki, H. Okumura, K. Hashimoto, K. Yoshikawa, Involvement of endogenously produced 1,25-dihydroxy Vitamin D3 in the growth and differentiation of human keratinocytes, Biochim. Biophys. Acta 1092 (1991) 311.
[41] D.D. Bikle, S. Pillai, E. Gee, Squamous carcinoma cell lines produce 1,25-dihydroxy Vitamin D, but fail to respond to its prodifferentiating effect, J. Invest. Dermatol. 97 (1991) 435.
[42] J.A. McLaughlin, L.C. Cantley, M.F. Holick, 1,25(OH)2D3 increased calcium and phosphatidylinositol metabolism in differentiating cultured human keratinocytes, J. Nutr. Biochem. 1 (1990) 81.
[43] Y. Yada, T. Ozeki, S. Meguro, S. Mori, Y. Nozawa, Signal transduction in the onset of terminal keratinocyte differentiation induced by 1-alpha,25-dihydroxy Vitamin D3: role of protein kinase C translocation, Biochem. Biophys. Res. Commun. 163 (1989) 1517.
[44] W. Tang, V.A. Ziboh, Agonist/inositol trisphosphate-induced release of calcium from murine keratinocytes: a possible link with keratinocyte differentiation, J. Invest. Dermatol. 96 (1991) 134.
[45] A.V. Ratnam, D.D. Bikle, J.K. Cho, 2:1,25-Dihydroxy Vitamin D3 enhances the calcium response of keratinocytes, J. Cell Physiol. 178 (1999) 188.
[46] S. Pillai, D.D. Bikle, M.J. Su, A. Ratnam, J. Abe, 1,25-Dihydroxy Vitamin D3 upregulates the phosphatidylinositol signaling pathway in human keratinocytes by increasing phospholipase C levels, J. Clin. Invest. 96 (1995) 602.
[47] Z. Xie, D.D. Bikle, Inhibition of 1,25-dihydroxy Vitamin-D-induced keratinocyte differentiation by blocking the expression of phospholipase C-gamma-1, J. Invest. Dermatol. 117 (2001) 1250.
[48] D.C. Ng, S. Shafaee, D. Lee, D.D. Bikle, Requirement of an AP-1 site in the calcium response region of the involucrin promoter, J. Biol. Chem. 275 (2000) 24080.
[49] D.D. Bikle, D. Ng, Y. Oda, K. Hanley, K. Feingold, Z. Xie, The Vitamin D response element of the involucrin gene mediates its regulation by 1,25-dihydroxyVitamin D3, J. Invest. Dermatol. 119 (2002) 1109.
[50] Z. Xie, L. Komuves, Q.C. Yu, H. Elalieh, D.C. Ng, C. Leary, S. Chang, D. Crumrine, T. Yoshizawa, S. Kato, D.D. Bikle, Lack of the Vitamin D receptor is associated with reduced epidermal differentiation and hair follicle growth, J. Invest. Dermatol. 118 (2002) 11.
[51] D.D. Bikle, S. Chang, D. Crumrine, H. Elalieh, M.Q. Man, O. Dardenne, Z. Xie, R.S. Arnaud, K. Feingold, P.M. Elias, Mice lacking 25OHD 1-alpha-hydroxylase demonstrate decreased epidermal differentiation and barrier function, J. Steroid Biochem. Mol. Biol. 89–90 (2004) 347.