The Osteoblast Transcription Factor Runx2 Is Expressed in Mammary Epithelial Cells and Mediates osteopontin Expression*

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Targeted deletion of the Runx2 gene in mice has demonstrated that Runx2 is a master regulator of osteoblast differentiation. Runx2 has therefore largely been regarded as a bone-specific transcription factor. Runx2+/− mice die shortly after birth and therefore the role of Runx2 in later developing tissues remains unclear. Here we show that the Runx2 protein is expressed in several mammary epithelial cell lines and in primary mammary epithelial cells. In addition, we have also found that it has a functionally important role in gene regulation. Osteopontin (OPN) is expressed in mammary epithelial cells during pregnancy and lactation and has been shown to have a role in mammary gland differentiation. Here we show that a Runx2 site in the OPN promoter is required for activation of the promoter in mammary epithelial cells. Moreover, dominant-negative Runx proteins can inhibit both activation of an OPN promoter reporter in transient transfections and expression of the endogenous OPN gene in mammary epithelial cells. Our data suggest, for the first time, that the osteoblast transcription factor Runx2 has a role in the normal regulation of gene expression in mammary epithelial cells.

Targeted deletion of the Runx2 gene in mice has demonstrated that Runx2 is a master regulator of osteoblast differentiation and is required for chondrocyte hypertrophy (1–3). Runx2 has therefore largely been regarded as a bone-specific transcription factor. Runx2+/− mice die shortly after birth and therefore the role of Runx2 in later developing tissues remains unclear. However, mice containing a targeted replacement of Runx2 with LacZ showed β-galactosidase activity in the epithelium of the nascent mammary gland (1). This appears to be the only other major site of Runx2 expression outside of the skeleton, suggesting that Runx2 has a role in gene regulation in the mammary gland.

Runx2 expression has recently been reported in metastatic mammary epithelial cells and not in normal human mammary cells (4). It was therefore proposed that expression of Runx2 in metastatic breast cancer cells is ectopic, and that the expression of Runx2 in these cells may explain the osteoblastic phenotype of human breast cancer cells that metastasize to the bone (4).

Here we describe two important findings that support a role for Runx2 in normal mammary epithelial cells. First, we show that Runx2 protein is expressed in mammary epithelial cell lines derived from normal mammary gland, non-metastatic mammary cancer cells and primary mammary epithelial cells. Second, Runx2 has a functionally important role in gene regulation in these cells. The osteopontin (OPN)1 gene is a known target gene of Runx2 in osteoblasts (5). OPN is also expressed in mammary epithelial cells during pregnancy and lactation and has been shown to have a role in mammary gland differentiation (6–8). OPN is a secreted integrin-binding extracellular matrix protein, which has several functions including stimulation of cell adhesion, cell signaling, cell migration, and protection against apoptosis (reviewed in Ref. 9). Since OPN is expressed in mammary epithelial cells we investigated the possibility that Runx2 might regulate OPN expression in these cells. We demonstrate that Runx2 from mammary epithelial cells binds a consensus recognition site in the OPN promoter and that this site is required for transcriptional activation in mammary epithelial cells. We also show that dominant-negative Runx proteins, and RNAi transcripts targeted against Runx2, can inhibit activation of the OPN promoter. Furthermore, expression of the endogenous OPN gene was inhibited by dominant-negative Runx proteins in mammary epithelial cells. Our data demonstrate that Runx2 is expressed in primary, non-metastatic and non-tumorigenic mammary epithelial cells. Moreover, we have shown, for the first time, that the osteoblast transcription factor Runx2 has a role in the regulation of a gene that is expressed in normal mammary epithelial cells.

EXPERIMENTAL PROCEDURES

RT-PCR—Total RNA was isolated from cells using the StrataPrep® Total RNA miniprep kit in accordance with the manufacturer's protocol (Stratagene). 2 μg of total RNA was reverse transcribed by incubation with 1 μl of 5 μM oligo(dT) primer at 70 °C for 10 min, after which 1 μl of dNTPs, 40 units of rRNasin® (Promega), 5 μl of reverse transcriptase buffer (HT Biotechnology), and 10.5 units of SUPER RT (HT Biotechnology) were added in a total volume of 25 μl. This was incubated at 42 °C for 1 h, 2 μl of the cDNA produced was used as a template for a PCR reaction. Amplification of Runx2 cDNA was achieved using primers Runx2P1 5′-ATTAGGGCGCATTCCTCATC-3′ and Runx2P2 5′-TGTAACTGTACCTGCTCCTTGAGAT-3′ (10). For amplification of OPN cDNA primers OP5′ 5′-TGACCCAGATCCTATAGCCC-3′ and OP5′ 5′-GGAGTGAAAGTGTCTGCTTG-3′ were used (11). For amplification of GAPDH cDNA primers GAPDHP1 5′-CAGTAGACCTCAGCCGAGGCTTCC-3′ and GAPDH2 5′-TTGTCATGGATGACCTTGGC-3′ were used. PCR amplifications were achieved by 30 cycles of, 95 °C for 1 min, 55 °C for 1 min, 68 °C for 2 min, with a final extension step at 68 °C for 5 min. Samples were electrophoresed on a 3% agarose gel, and relative amounts of DNA were quantified using Quantity One software (BioRad).

Immunoblotting—Nuclear extracts were prepared as previously described (12), equal amount of which were electrophoresed on a 12%
SDS-polyacrylamide gel. The proteins were transferred onto a nitrocellulose membrane using a trans-blot 5 D. cell (Bio-Rad). After blocking with TBS-0.05% Tween 20, 5% dried milk, the membrane was incubated with a polyclonal anti-Rux2 antibody raised against a Rux2-specific peptide, diluted 1:4000 (Oncogene research products). Following subsequent washes the membrane was incubated with horseradish peroxidase conjugated goat anti-rabbit antibody, diluted 1:2000 (BD Pharmaningen). Immune complexes were detected using Supersignal West Dura Extended Duration Substrate (Pierce) and visualized using a Bio-Rad Fluor-S multi-imager.

**Electrophoretic Mobility Shift Assays (EMSAs):** Oligonucleotides were radiolabeled with [α-32P]dCTP using Klenow fragment according to standard protocols (13). The following oligonucleotides, RuxTop 5'-CTAGAAGCTGGGCTGTCGGTG-3' and RunxBot 5'-CTAGACGGCAGCTGGTCAAGT-3' contain a Runx-binding site (Runx) and were used in EMSAs (14). The following oligonucleotides were used in EMSAs investigating the Runx site present at 72 bp to 128 bp in the OPN promoter. Nuclear extract from HC11 cells was incubated with radiolabeled OPN oligonucleotide in the presence or absence of 100-fold molar excess of competitor DNA as indicated above the lanes. The identity of each complex is indicated. 

**Plasmid Constructs**—The OPN promoter was amplified by nested PCR from genomic DNA derived from HC11 cells using the following oligonucleotides. First-round amplification primers were OPN5 P1 5'-CGGCAAAGCTTCCGAGAATGCCTGCCGCAG-3' and OPN3 P2 5'-CTAGGATCTTCTCTCTGTTTTAAAGGAGAAAACC-3'. The PCR product was ligated into the pGADD153i vector and used to generate the OPN promoter constructs. The following oligonucleotides were ligated into the pGADD153i vector according to the manufacturer's instructions. The RuxTop oligonucleotide was radiolabeled with [α-32P]dCTP using Klenow fragment according to standard protocols (13). The following oligonucleotides, RuxTop 5'-CTAGAAGCTGGGCTGTCGGTG-3' and RunxBot 5'-CTAGACGGCAGCTGGTCAAGT-3' contain a Runx-binding site (Runx) and were used in EMSAs (14). The following oligonucleotides were used in EMSAs investigating the OPN promoter. The identity of each complex is indicated.

**Runx2 in Mammary Epithelial Cells**—The pGADD153i vector was kindly provided by Dr. Alan Dickson.2 The RHD plasmid was constructed by insertion of the Runx1 Runx domain into pCMV4. Runx2 and AML-1/ETO were expressed using pCMV-OSP2 and pCMVAML-1/ETO respectively (10, 16).

**Cell Culture**—HC11 cells were maintained in RPMI 1640 with 10% fetal bovine serum, penicillin/streptomycin (Sigma) and 10 mM 125O2D3 (Sigma). HC11 cells were cultured in the presence or absence of 10 nM 1,25(OH)2D3 (Sigma). HC11 cells were cultured in DMEM with 4.5g/liter glucose, 10% FBS, 1 mM sodium pyruvate, and 1 mM sodium pyruvate (20). All cells were maintained at 37 °C in 5% CO2. Primary mammary epithelial cells were isolated from day 17 pregnant mice as previously described (21).

**Transfections**—All cell lines were transfected in 24-well plates using LipofectAMINE 2000 transfection reagent (Invitrogen), according to the manufacturer's instructions. A total of 800 ng of DNA was used in each transfection. Each transfection contained 380 ng of the indicated reporter construct and 40 ng of pRLSV40 (Promega), which was used to normalize for transfection efficiency. Five hours post-transfection the transfection mixture was removed and replaced with normal cell media. Twenty-four hours post-transfection the cells were lysed and the luciferase activity determined using the dual luciferase reporter assay system (Promega) according to the manufacturer's recommendations. All transfections were performed in triplicate, and data is presented as mean values with standard deviation. All values are relative to the activity of the pGL3-Basic reporter. For analysis of the endogenous OPN expression 1 × 106 HC11 cells were transfected with 1 μg of pGFP and 1 μg of the stated expression construct, using LipofectAMINE2000 in accordance with the manufacturer's instructions (Invitrogen). Twenty-four hours post-transfection the cells were harvested and FACS sorted and the viable transfected cells were collected. Total RNA was harvested from 3.6 × 106 of these cells, and was analyzed by RT-PCR. For RNAi-expressing plasmids HC11 cells were transfected with 0.8 μg of pSUPER, pRunx2i, or pGADD153i. Forty-eight hours post-transfection the cells were further transfected with a total of 1 μg of DNA. Each transfection contained 380 ng of either pGL3-Basic or pGL3Basic-OPN, 40 ng of pRLSV40, which was used to normalize for transfection efficiency, and 600 ng of either pSUPER, pRunx2i, or pGADD153i.

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2 A. Dickson, unpublished data.
as indicated. The cells were lysed 48 h after the second transfection and luciferase activity was determined.

RESULTS

Runx2 Protein Is Expressed in Mammary Epithelial Cells—HC11 cells are mammary epithelial cells derived from mice in mid-pregnancy, they maintain the ability to express milk proteins in response to lactogenic hormones and are an established model for studying gene expression in non-tumorigenic mammary epithelial cells (22–26). HC11 cells were therefore used to investigate the expression of Runx2. RT-PCR was performed on total RNA derived from HC11 cells and the pre-osteoblast cell line MC-3T3, which is known to express Runx2 (Fig. 1A; Ref. 20). Runx2 transcripts were detected in HC11 and MC-3T3 cells (Fig. 1A, lanes 2 and 4) but not in HeLa cells, which do not express Runx2 (data not shown; Ref. 16).

A further control for specific Runx2 transcript expression was performed by treatment of HC11 cells with the vitamin D3 hormone, 1,25(OH)2D3. Stimulation of osteoblasts with 1,25(OH)2D3 has been shown to abolish Runx2 expression (10). Since HC11 cells are known to express functional vitamin D3 receptors we tested whether 1,25(OH)2D3 also down-regulates Runx2 expression in HC11 cells (Fig. 1B; Ref. 27). Treatment of HC11 cells with 1,25(OH)2D3 almost completely abolished Runx2 transcripts in HC11 cells but had no effect on GAPDH expression (Fig. 1B), indicating that Runx2 expression is regulated in a similar manner by 1,25(OH)2D3 in mammary epithelial cells and osteoblasts.

To establish if functional Runx2 protein is present in HC11 cells, nuclear extracts were incubated with the Runx2-binding site from the OPN promoter and the formation of specific complexes was determined by EMSA. Specific binding activity was first determined by a competition assay. A double-stranded radiolabeled oligonucleotide encompassing the Runx site at position −128 to −123 bp in the OPN promoter (RunxOPN, Fig. 1C) was used in the competition assays with a known Runx-binding site (Runx, Fig. 1C) and a mutant form of the OPN Runx site (MRunxOPN, Fig. 1C). When the RunxOPN site was incubated with nuclear extracts from HC11 cells a specific retarded complex was observed (Fig. 1D, lane 1). This complex was abolished in the presence of 100-fold molar excess of either the wild-type RunxOPN site or an alternative Runx-binding site (Fig. 1D, lanes 2 and 3). In contrast, the mutated OPN Runx site did not abrogate binding (Fig. 1D, lane 4). These data demonstrate that the Runx site in the OPN promoter can be specifically bound by a factor in mammary epithelial cells.

To establish if the Runx protein present in HC11 cells was Runx2 the RunxOPN probe was incubated with nuclear extracts in the presence of a Runx2-specific antibody (Fig. 1E). The entire specific complex was supershifted by the anti-Runx2 antibody (Fig. 1E, lane 3). In contrast no supershifted complex was observed when the Runx1 antibody was used as a control (Fig. 1E, lane 2). Nuclear extracts from Jurkat T cells, which are known to express Runx1, were used to confirm that the anti-Runx1 antibody was functional (Fig. 1E, lanes 4–6). These results clearly show that Runx2 is expressed in HC11 cells and that it specifically binds to the Runx site in the OPN promoter.

Runx2 Is Expressed in Mammary Cancer Cell Lines and Primary Mammary Cells—To determine if Runx2 is expressed in other mammary epithelial cells, immunoblotting was performed on nuclear extracts from several cell lines (Fig. 2A). UMR-106 cells are an osteosarcoma cell line known to express Runx2 and were used as a positive control (Fig. 2A, lane 5; Ref. 28). The 64kDa Runx2 isoform was detected in nuclear extracts derived from all the mammary epithelial cell lines tested (Fig. 2A, lanes 1–4). A slightly larger protein was also detected in MCP-7 and HC11 cells, which may correspond to a phospho-

![Image](Runx2 in Mammary Epithelial Cells)
Fig. 3. Runx2 activates the OPN promoter in mammary epithelial cells. A, schematic diagram illustrating the OPN promoter reporters used in this study. The 5′-sequence of the mouse OPN gene (OPNwt), from −807 bp to +72 bp is shown ligated upstream of the luciferase cDNA (unfilled box) (5, 11). The location and sequence of the Runx site is shown (filled box). The mutated reporter is also shown (OPNmut). B, HC11, HeLa, and UMR-106 cells were transfected with 360 ng of the luciferase reporter constructs containing, either the OPN wild-type promoter (OPNwt), black bars, or the Runx site mutant (OPNmut), gray bars. C, HC11 cells were transfected with 360 ng of either OPNwt (black bars) or OPNmut (gray bars) in the presence of increasing amounts of Runx2 (0, 20, 40, 200, and 400 ng). All transfections were performed in triplicate; luciferase activities are presented as mean values with S.D. All values are relative to the activity of the pGL3-Basic reporter.

Fig. 4. Dominant-negative forms of Runx factors repress OPN expression in mammary epithelial cells. A, HC11 cells were transfected with 360 ng of the luciferase reporter plasmids OPNwt (black bars) or OPNmut (gray bars) in the presence of increasing amounts of AML-1/ETO (0, 40, 100, 200, and 400 ng). B, MCF-7 cells were transfected with either OPNwt (black bars) or OPNmut (gray bars) in the presence or absence of AML-1/ETO (400 ng) as indicated. C, HC11 cells were transfected with 360 ng of either OPNwt or OPNmut in the presence or absence of increasing amounts of RHD (0, 40, 100, 200, and 400 ng). D, HC11 cells were transfected with the luciferase reporter plasmid OPNwt in the presence of pSUPER, pGADD153i, or pSUPER-Runx2i as indicated. All transfections were performed in triplicate; luciferase activities are presented as mean values with S.D. All values are relative to the activity of the pGL3-Basic reporter.

breast cancer cell lines (MDA-MB-231) but is also clearly expressed in non-metastatic breast cancer cells (MCF-7), non-tumorigenic (HC11) and primary mammary epithelial cells.

Runx2 Contributes to Transcriptional Activation of the OPN Promoter in Mammary Epithelial Cells—Having shown that Runx2 is expressed in mammary epithelial cells we next sought to establish whether it has a functionally important role in gene regulation. OPN is expressed in mammary epithelial cells during pregnancy and lactation and has been shown to have a role in mammary gland differentiation (6–8). To determine whether the Runx site in the OPN promoter was required for transcriptional activation in mammary epithelial cells three different cell lines, HC11, HeLa, and UMR-106, were transfected with an OPN promoter reporter plasmid, containing either the wild-type promoter (pGL3B-OPNwt, Fig. 3A) or one in which the Runx site was mutated (pGL3B-OPNmut; Fig. 3A). Comparison of the activity of the wild-type OPN promoter in the three cell types revealed that the basal level of activity was similar in the HC11 and UMR-106 cells but significantly less in HeLa cells (Fig. 3B). When the Runx site was mutated the activity of the promoter in HC11 and UMR-106 cells was reduced by ~50%; this activity is equal to that observed with the wild-type promoter in HeLa cells. In contrast, the activity of the mutated promoter in HeLa cells was not significantly affected (Fig. 3B). These data clearly demonstrate that the Runx site is essential for full transcriptional activity of the OPN promoter in HC11 cells.

To test whether Runx2 is able to activate the OPN promoter in mammary epithelial cells, HC11 cells were co-transfected with the OPN reporter plasmids and increasing amounts of a Runx2 expression plasmid (Fig. 3C). Heterologous expression of Runx2 increased the transcriptional activity of the wild-type OPN reporter at all levels tested. In contrast, mutation of the Runx site in the OPN promoter rendered the promoter unresponsive to Runx2. These observations demonstrate that Runx2 can act as a transcriptional activator of the OPN gene in HC11 cells.

Dominant-negative Runx Proteins Inhibit OPN Promoter Activity—To determine whether dominant-negative Runx proteins can repress the activity of the OPN promoter via the Runx
site in mammary epithelial cells we co-transfected HC11 cells with the OPN promoter reporters and a plasmid encoding AML-1/ETO. AML-1/ETO is a repressive form of Runx1 arising from the (8; 21) translocation and is often used to specifically inhibit transcriptional activation via Runx sites (30–33, 16). When the wild-type OPN promoter was co-transfected with the AML-1/ETO-expressing construct, transcriptional activity of the promoter was inhibited with a maximum 6.2-fold inhibition when AML-1/ETO-expressing construct, transcriptional activity of the promoter was inhibited with a maximum 6.2-fold inhibition with 400 ng of AML-1/ETO expression plasmid (Fig. 4). The inhibitory effect of AML-1/ETO was not particular to the OPN promoter. AML-1/ETO is a repressive form of Runx1 arising from the (8; 21) translocation and is often used to specifically inhibit transcriptional activation via Runx sites (30–33, 16). When the wild-type OPN promoter was co-transfected with the AML-1/ETO-expressing construct, transcriptional activity of the promoter was inhibited with a maximum 6.2-fold inhibition observed with 400 ng of AML-1/ETO expression plasmid (Fig. 4A, OPNwt). When AML-1/ETO was co-expressed with the OPN promoter containing the mutated Runx site, we observed maximum inhibition of only 2-fold (Fig. 4A, OPNmut). AML-1/ETO also repressed the OPN promoter in the human mammary epithelial cell line MCF-7, demonstrating that this effect is not restricted to HC11 cells (Fig. 4B).

The inhibitory effect of AML-1/ETO was not particular to this oncoproteinic protein as we observed almost identical inhibition when an isolated Runx-domain (RHD), which is known to bind to the endogenous OPN promoter by ChIP assay but were unable to do so in HC11 cells or in control osteoblasts, although we have previously shown Runx1 bound to an endogenous promoter (data not shown; Ref. 33). One possible explanation for this is that the antibody epitope is inaccessible when Runx2 is bound to the promoter, where it is likely to be part of a larger nucleoprotein complex. We therefore determined whether expression of the endogenous OPN gene could be inhibited by dominant-negative Runx proteins. HC11 cells were co-transfected with a GFP expression plasmid and either the AML-1/ETO or RHD expression plasmids (Fig. 5). Viable transfected cells were isolated by FACS. RNA was prepared from these cells and RT-PCR performed. The level of endogenous OPN mRNA expression was decreased by 3.4-fold in cells transfected with either the AML-1/ETO or the RHD expression plasmids (Fig. 5). Taken together with the EMSA and RNAi data this strongly suggests that Runx2 contributes to the transcriptional regulation of the endogenous OPN gene in mammary epithelial cells (Fig. 5). Taked together with the EMSA and RNAi data this strongly suggests that Runx2 contributes to the transcriptional regulation of the endogenous OPN gene in mammary epithelial cells.

DISCUSSION

The reported phenotypic defects observed in Runx2-deficient mice are largely limited to the skeleton, Runx2 has therefore been regarded as a transcriptional regulator specific to osteoblasts and chondrocytes (1–3). In this report we have clearly demonstrated that Runx2 is also expressed in mammary epithelial cells. We observed Runx2 expression in different mammary epithelial cell lines and in primary mammary epithelial cells. We have also shown that Runx2 has functional significance in mammary epithelial cells, by demonstrating its role in OPN gene expression. These findings therefore indicate that, in addition to its role in skeletal development, Runx2 also has a role in regulating gene expression in the mammary gland.

Our finding that Runx2 is expressed in primary mammary epithelial cells is consistent with the observation that mice containing a targeted replacement of Runx2 with LacZ showed β-galactosidase activity in the epithelium of the nascent mammary gland (1). However, this contrasts with a recent report that demonstrated expression of Runx2 in metastatic breast cancer cells which metastasise to the bone, and not in normal human mammary cells (4). The authors proposed that expression of Runx2 in metastatic mammary epithelial cells is ectopic and that this may explain the osteoblastic phenotype of human breast cancer cells that metastasise to the bone. However, we have clearly shown that in addition to expression in metastatic cells, Runx2 is expressed in primary, non-metastatic and non-tumorigenic mammary epithelial cells. The differences between Runx2 expression in normal mammary epithelial cells may reflect a species difference; we examined Runx2 expression in mice whereas the previous report analyzed human cells (4). Alternatively, Runx2 expression may be regulated throughout mammary gland development; we obtained primary mammary epithelial cells from day 17 pregnant mice when we would expect OPN to be expressed. However, it is not clear at which stage of development the human breast cells were obtained (4) and that this may explain the osteoblastic phenotype of human breast cancer cells. However, we have clearly shown that in addition to expression in metastatic cells, Runx2 is expressed in primary, non-metastatic and non-tumorigenic mammary epithelial cells. The differences between Runx2 expression in normal mammary epithelial cells may reflect a species difference; we examined Runx2 expression in mice whereas the previous report analyzed human cells (4). Alternatively, Runx2 expression may be regulated throughout mammary gland development; we obtained primary mammary epithelial cells from day 17 pregnant mice when we would expect OPN to be expressed. However, it is not clear at which stage of development the human breast cells were obtained (4). Regardless of any potential species differences in Runx2 ex-
pression we have shown that non-metastatic human mammary epithelial cells (MCF-7) also express Runx2. If Runx2 does contribute to the bone metastatic potential of mammary epithelial cells then it is possible that Runx2 activity is aberrantly regulated in these cells.

The mammary gland is an estrogen responsive tissue and estrogen has been shown to enhance Runx2 activity in osteoblasts via the direct interaction of the estrogen receptor with Runx2, independently of changes in Runx2 expression (34). Similarly, we have found that Runx2 DNA-binding activity was unaltered when HC11 cells were treated with estradiol in pheno red-free media.3 In addition we did not observe an increase

unaltered when HC11 cells were treated with estradiol in phenol red-free media.3 In addition we did not observe an increase

However, we have shown that Runx2 activity is aberrantly regulated via Runx2.3 How-

ever, we have showed that Runx2 has a functional role in the regulation of gene expression in mammalian epithelial cells. Moreover, we have shown that Runx2 has a functional role in the regulation of gene expression in mammary epithelial cells.

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REFERENCES

1. Otto, F., Thornell, A. P., Crompton, T., Denzel, A., Gilmore, K. C., Rosewell, I. R., Stamp, G. W., Beddington, R. S., Mundlos, S., Olsen, B. R., Selby, P. B., and Owen, M. J. (1997) Cell 89, 765–771.

2. Komori, T., Yagi, H., Nomura, S., Yamaguchi, A., Sasaki, K., Deguchi, K., Shimizu, Y., Bronson, R. T., Gao, Y. H., Inada, M., Sato, M., Okamoto, R.,

3. K. Inman and P. Shore, unpublished observation.

3 C. K. Inman and P. Shore, unpublished observation.
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