

## The Oncogenic Role of miR-155 in Breast Cancer

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### Abstract

miR-155 is an oncogenic miRNA with well described roles in leukemia. However, additional roles of miR-155 in breast cancer progression have recently been described. A thorough literature search was conducted to review all published data to date, examining the role of miR-155 in breast cancer. Data on all validated miR-155 target genes was collated to identify biologic pathways relevant to miR-155 and breast cancer progression. Publications describing the clinical relevance, functional characterization, and regulation of expression of miR-155 in the context of breast cancer are reviewed. A total of 147 validated miR-155 target genes were identified from the literature. Pathway analysis of these genes identified likely roles in apoptosis, differentiation, angiogenesis, proliferation, and epithelial–mesenchymal transition. The large number of validated miR-155 targets presented here provide many avenues of interest as to the clinical potential of miR-155. Further investigation of these target genes will be required to elucidate the specific mechanisms and functions of miR-155 in breast cancer. This is the first review examining the role of miR-155 in breast cancer progression. The collated data of target genes and biologic pathways of miR-155 identified in this review suggest new avenues of research for this oncogenic miRNA. *Cancer Epidemiol Biomarkers Prev*; 21(8); 1236–43. ©2012 AACR.

### Introduction

miRNAs are small noncoding RNAs that control expression of target genes by either inhibiting protein translation or directly targeting mRNA transcripts of target genes for degradation (1). Each miRNA has a specific seed sequence 7 to 8 nucleotides long, which directly binds to complementary sequences in regulatory regions of target genes. These binding regions are often in the 3'-untranslated region (3'-UTR) of target genes, but increasingly are being reported in other noncoding regions such as promoter or intronic regions (2). The short length of the seed sequence facilitates the targeting of many transcripts by a single miRNA (3). Some estimates suggest that 30% of all eukaryotic genes are regulated by miRNAs (4, 5). miR-155, a miRNA widely reported to be involved in lymphoma, is also now emerging to have a role in the progression of solid cancers (6). This review will focus on the miRNA miR-155, and its role in breast cancer.

miRNAs were discovered in 1993 when the *C. elegans* *lin-4* gene, which is transcribed but not translated, was

found to regulate levels of LIN-14 protein (7, 8). Since this discovery, there have been over 500 miRNAs described, regulating a wide range of genes and cellular processes, although the total predicted number of unique miRNAs encoded by the human genome is estimated to be more than 1,000 (9). Many of these miRNAs are organized as gene clusters and transcribed as multicistronic messages—for example, the *MIRH1* gene encodes 6 different miRNAs (10). The transcription and processing of miRNAs has been well characterized and is depicted in Fig. 1 using miR-155 as an example. miRNAs originate from an approximately 70 nucleotide RNA hairpin pre-miRNA processed from the RNA transcript of the host gene (11); in the case of miR-155, the host gene *BIC*. The pre-miRNA is typically cleaved by the Drosha and Dicer exonucleases into an approximately 22 nucleotide RNA duplex. One strand of the duplex becomes the mature miRNA and is usually the functional, regulatory unit (12, 13), whereas the other is designated miR\* and is usually degraded. The mature miRNA is loaded into Argonaute proteins, forming the RNA Induced Silencing Complex (RISC). The mature miRNA may then bind to its target by partial complementarity of target gene mRNA and either inhibit translation or cause degradation of the mRNA.

The miR-155 host gene, *BIC*, was first described in 1989 and postulated to be involved in the progression of lymphoma (14). In 2002, Lagos-Quintana and colleagues identified miR-155 as a regulatory RNA (15). Subsequently, studies have focused on the roles of miR-155 in lymphoma (16–19) and also in viral infection, cardiovascular disease, and solid cancers (6, 20–22). miR-155 has more than 400 predicted gene targets (23) and more than 100

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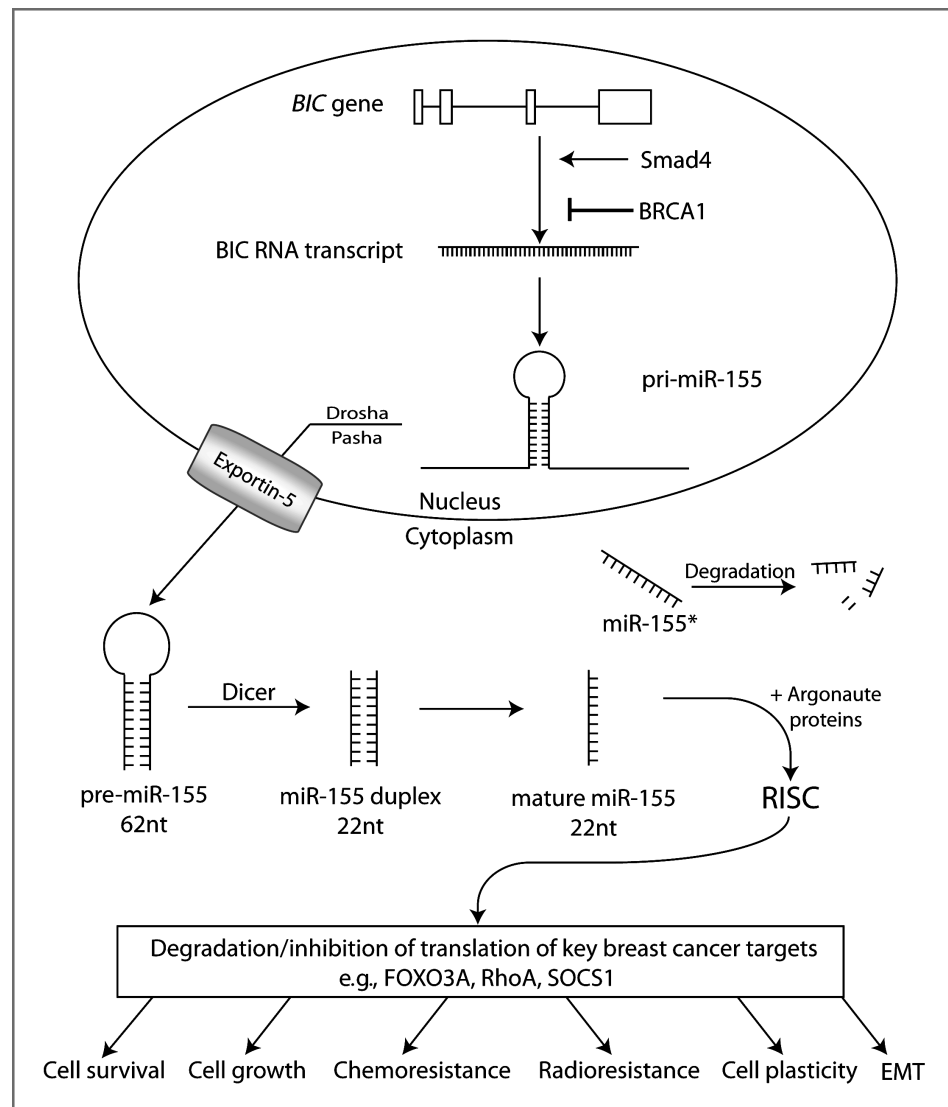
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**Figure 1.** Cellular processing and downstream effects of miR-155 in breast cancer. The pri-miR-155 RNA hairpin transcript is processed from the RNA transcript of the *BIC* gene. Transcription of *BIC* is promoted by Smad4 and inhibited by BRCA1. After processing by Drosha, Pasha, Exportin-5, and Dicer, the mature miR-155 forms a complex with Argonaute proteins called the RISC, to inhibit the translation of miR-155 target mRNAs, such as RhoA, FOXO3A, and SOCS1. The inhibition of target genes by miR-155 in breast cancer can cause such effects as an increase in EMT, cell plasticity, cell survival, growth, chemoresistance, and radioresistance.



confirmed *bona fide* targets. There is now an emerging role of miR-155 in breast cancer progression (20, 21, 24), which is the focus of this review.

### Clinical Relevance of miR-155 in Breast Cancer

Studies show the expression level of miR-155 is upregulated in breast cancer with high levels of miR-155 associated with clinicopathologic markers, tumor subtype, and poor survival rates, summarized in Table 1 (21, 25–34). Of 29 miRNAs found to be dysregulated in breast cancer, the majority were downregulated, with only miR-155 and miR-21 significantly upregulated (25). Expression levels of 15 of these dysregulated miRNAs independently predict the invasive potential of breast tissue samples (25). A small microarray study of 8 fresh breast tumor samples found miR-155 was upregulated in the breast tumors compared with normal adjacent

tissue (34). In a larger study, 62 breast carcinomas were analyzed to determine miR-155 levels. Of 17 noninvasive tumors, only 2 (12%) exhibited a high level of miR-155 expression. Conversely, 41 of the 45 invasive tumors (91%) displayed miR-155 upregulation (32). In a further study, expression levels of *FOXO3A*, a miR-155 target gene, was determined in 77 primary breast tumors, 38 recurrent tumors, and 11 normal tissue samples. Results showed that miR-155 was upregulated and *FOXO3A* downregulated in a majority of primary tumors, and also that high miR-155 and low *FOXO3A* expression was associated with recurrent tumors after radiotherapy or chemotherapy (21). These studies linked miR-155 expression to both invasiveness and recurrence of breast tumors and showed that expression levels of miR-155 and its specific target genes are of potential clinical prognostic value.

**Table 1.** Summary studies examining miR-155 expression in breast cancer

miR-155	Tissue type	Ref.
↑ in breast cancer	76 Breast tumor	(25)
	10 Normal breast	
↑ in breast cancer	363 Breast tumor	(26)
	177 Normal breast	
↑ in ER <sup>-</sup> tumors	93 Breast tumor	(27)
	5 Normal breast	
↑ in malignant breast tissue	34 Breast tumor	(28)
	6 Normal breast	
↑ in PR <sup>+</sup> tumors	Serum-13 breast cancer patients, 8 healthy patients	(29)
↑ in grade II and III tumors	Tumor, normal adjacent tissue, and serum from 68 breast cancer patients	(30)
↑ in ER <sup>-</sup> PR <sup>-</sup> tumors	Tissue and serum from 40 healthy patients	
Associated with higher tumor grade, advanced tumor stage, lymph node metastasis	92 Breast tumor and normal adjacent tissue	(31)
↑ in 41 of 45 invasive	45 Invasive breast tumor	(32)
↑ 2 of 17 noninvasive tumors	17 Noninvasive breast tumor	
↑ in 55 breast tumors	77 breast tumor	(21)
↑ 31 recurrent tumors	11 Normal breast	
	38 Recurrent breast tumor	
↑ in breast metastases	13 Breast tumor and paired metastasis	(33)
↑ in tumors	8 Breast tumor and normal adjacent tissue	(34)

In a robust study of lung, stomach, prostate, colon, pancreatic tumors, and 363 breast tumors, Volinia and colleagues globally compared miRNA expression levels in multiple tumor and pooled normal tissue samples to identify dysregulated miRNAs in tumor samples. Comparisons of normal and tumor tissue derived from each individual tissue showed that miR-155 expression was upregulated in breast, colon, and lung cancers. Interestingly, miR-155 was one of only 2 miRNAs (the other being the miR-200 family) found to be upregulated in both breast and lung cancer, implying that these miRNAs may be part of a common mechanism in the development of cancer in these organs (26).

miR-155 expression levels have been shown to be associated with metastasis events and invasive properties of breast cancer. In one study, increased miR-155 expression was associated with high tumor grade, advanced stage, and lymph node metastasis (31). Disease-free and overall survival were also negatively correlated with miR-155 levels, further showing the potential of miR-155 as a miRNA of clinical interest. These findings were further supported by 2 studies involving microarray analyses of formalin-fixed, paraffin-embedded breast cancer samples, which found that miR-155 expression was upregulated in metastases (28, 33).

Because miR-155 is associated with poor prognosis and/or metastasis, a correlation of miR-155 levels with breast cancer clinicopathologic markers would be expected. Analysis of 93 breast cancers for both miRNA levels alongside mRNA levels, to classify tumor subtypes, showed miR-155 levels were significantly upregulated in

basal-like tumors and in estrogen receptor negative (ER<sup>-</sup>) tumors (27). The correlation with basal-like tumors has particular clinical relevance because of the poor prognosis of this tumor subtype.

Studies have investigated whether serum samples could be used to identify aberrant miRNA expression levels in breast cancer patients. In a small study of 21 patients, Zhu and colleagues found that multiple miRNAs could be detected in sera, and the miRNA levels correlated with the levels in tissue samples (29). The expression of miR-155 was higher in the serum of PR<sup>+</sup> breast cancer patients than in the serum of PR<sup>-</sup> patients (29). Further studies confirmed these findings, with a significant correlation ( $R^2 = 0.853$ ) between miRNA levels in fresh breast cancer tissue and matched serum samples (30). They confirmed that miR-155 was upregulated in breast cancer and also that high miR-155 was associated with grade II and III tumors and ER<sup>-</sup> and PR<sup>-</sup> tumors (30). The detection of miR-155 expression levels in serum is a potential clinical prognostic indicator of tumor grade and hormone receptor status. The relationship of PR status and miR-155 expression is unresolved, with 2 studies reporting contradictory results (27, 29). The topic of serum miRNAs is also somewhat controversial, with some studies suggesting that serum miRNA levels are robust (35, 36), and others claiming that the miRNAs often used as normalization controls are highly variable in sera samples and thus miRNA quantification in sera is not reproducible (37). This suggests analysis of serum alone is not sufficient to determine whether miR-155 is differentially expressed.

Because the number of samples in these studies is generally low, resolution requires a more robust study.

Taken together, these studies showed that miR-155 expression is upregulated in breast cancer, consistent with its status as an oncomiR, and is associated with more aggressive breast tumors. However, the relationships between miR-155 and clinicopathologic markers, such as ER and PR status and tumor subtype, is inconsistent, probably because of small sample sizes and methodologic aspects. For example, the upregulation of miR-155 expression in PR<sup>+</sup> tumors was only identified in one study of a small number of samples (29). Further studies are required to confirm and elucidate the basis of the relationship between miR-155 and hormone receptor status.

### Functional characterization of miR-155 oncogenic activities in breast cancer

An important step in determining the clinical significance of miR-155 is to determine whether high expression levels are causally related to the development of breast cancer. *In vitro* effects of altering miR-155 expression levels were assessed in a panel of breast cancer cell lines (21). miR-155 expression was inhibited by anti-miR in HS578T cells. An anti-miR is a 2'-O-methyl oligoribonucleotide that inhibits the action of an miRNA. One proposed mechanism for anti-miR action is antisense binding to the mature miRNA positioned in the RISC (38). The HS578T cell line expresses high levels of endogenous miR-155, and anti-miR-155 application resulted in cell-cycle arrest and induction of apoptosis, implicating miR-155 in these processes (21). Conversely, ectopic overexpression of miR-155 in BT474 cells, which express very low levels of endogenous miR-155, promoted cell proliferation and survival and also improved chemoresistance (21). Taken together, these findings showed that miR-155 has a role in cell proliferation and apoptosis, 2 cellular processes frequently aberrant in cancer. Similar results have also been reported in breast cancer cell lines MDA-MB-231 and MCF-7 in which ectopic miR-155 overexpression increased proliferation, whereas inhibition of miR-155 expression by a specific anti-miR inhibits proliferation and increases radiosensitivity of cells *in vitro* (20, 31).

Xenografted human breast cancer cells in immunodeficient mice provide *in vivo* confirmation of miR-155 as an oncomiR. Xenografts of MDA-MB-231 cells showed reduced tumor volumes compared with control xenografts when anti-miR-155 is expressed, whereas overexpression of miR-155 accelerated tumor growth (20). Similarly, a xenograft of MDA-MB-468 cells, with low endogenous miR-155 expression, showed accelerated tumor growth when miR-155 was overexpressed (24). In the same study, knockdown of miR-155 in an orthotopically transplanted mouse tumor cell line inhibited tumor growth (24). Contrary to this, a recent study using the 4T1 mouse mammary model showed that miR-155 had no effect on growth of the primary tumor (39).

Although numerous studies show that miR-155 is upregulated in human breast cancer, the cause of aberrant miR-155 levels is not well characterized. TGF $\beta$  treatment of NMuMG cells results in significant upregulation of miR-155 and an epithelial to mesenchymal transition (EMT; ref. 32). TGF $\beta$  is known to drive EMT, in which immobile epithelial cells alter their morphology to become motile mesenchymal cells to promote invasion (40) and, consequently, cancer progression (41, 42). In NMuMG cells, Smad4, a key signaling molecule in the TGF $\beta$  pathway, can bind to the *BIC* promoter and enrich miR-155 expression levels, thereby augmenting the TGF $\beta$  EMT process (32). Knockdown of miR-155 in NMuMG cells by anti-miR suppressed and ectopic overexpression of miR-155 enhanced TGF $\beta$ -mediated EMT (32). Furthermore, a key molecule in EMT, *RhoA*, is a target of miR-155, and expression of *RhoA* is reduced when miR-155 is ectopically expressed. When *RhoA* was expressed without its 3'-UTR (containing the miR-155 seed sequence), the EMT phenotype caused by miR-155 was abrogated (32). The ability to reverse a severe phenotypical change by reexpressing just one of the targets of miR-155 alludes to a potential therapeutic approach. Many miRNAs are known to have a role in metastasis and EMT (43); so in light of these findings, it is plausible that the basis of miR-155 in promoting breast cancer, in particular, the higher grade invasive breast cancers, is from the promotion of EMT. However, the findings from the 4T1 mouse model (39) contradict the findings in the NMuMG cell line (32). Unfortunately, both of the cell lines are of mouse origin. A miR-155 target gene in a mouse model will not necessarily be a target gene in humans, as the 3'-UTR region of transcripts is a common location for miRNA seed sequences, and is not highly conserved between mice and humans. These conflicting results call into question the suitability of using a mouse-specific model for an miRNA study.

### Regulation of miR-155 expression

Perhaps the most remarkable recent finding in relation to the role of miR-155 in breast cancer is the involvement with *BRCA1*. *BRCA1*, the breast cancer susceptibility gene, is involved in DNA damage repair and cell-cycle progression. Mutations of *BRCA1* are associated with a high risk of developing breast cancer (24). In a recent study, mouse embryonic stem cells expressing the R1699Q *BRCA1* underwent spontaneous differentiation. The mutant cells displayed high levels of miR-155, and overexpression of miR-155 in *BRCA1* wild-type cells gave a similar phenotype to the mutant, indicating that *BRCA1* was acting through miR-155 (24). In mice, a loss of functional *BRCA1* protein results in miR-155 upregulation. These results were recapitulated in human cell lines, in which *BRCA1* deficient cells have 50-fold higher miR-155 levels compared with those with functional *BRCA1* (24). Furthermore, the transient overexpression of *BRCA1* protein reduces expression of miR-155. In clinical samples, it was found that miR-155 levels were 2- to 6-fold

higher in *BRCA1*-mutant tumors (24). The mechanism of *BRCA1* regulation of miR-155 was through direct binding of *BRCA1* protein to the miR-155 promoter. This, in turn, recruits histone deacetylase (HDAC) to repress the expression of *BIC* and thus miR-155 (24). This close association with the breast cancer susceptibility gene reinforces the importance of miR-155 in breast cancer.

### Target genes of miR-155

The function of miRNAs are limited to inhibition of their target mRNA and consequent effects on cellular processes. miR-155 clearly has a role in breast cancer, and understanding this role requires the identification of critical miR-155 target genes.

TargetsScan is an *in silico* prediction software commonly used to identify putative target genes of particular miRNAs by alignment of the 7 or 8 nucleotide seed sequence with the 3'-UTR of 30,858 human transcripts based on conservation between human and mouse sequences (23). TargetsScan version 6 predicts 440 miR-155 targets (23, 44) based on sequence homology and conservation. Confirmation of these potential targets requires validation *in vitro*. To this end, we conducted a literature search to identify published validated miR-155 target genes. A target was defined as validated when there was a specific luciferase 3'-UTR reporter assay, which defines whether miR-155 directly targets the transcript, together with at least one other quantitative method, such as quantitative reverse transcriptase PCR or Western blot analysis, to assess the repression of the expression levels of the endogenous target gene.

Supplementary Table S1 displays a comprehensive list of 147 validated target genes identified in a wide range of

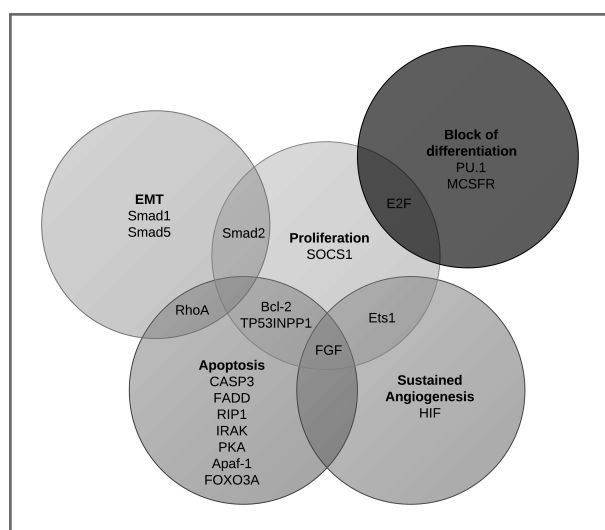
miR-155 studies (45–87), and their prediction status by TargetsScan. A total of 103 target genes (including 11 target genes validated in other studies) were identified in a single high-throughput next-generation sequencing study and validated by luciferase reporter assay (50). The remaining 44 target genes and their method of validation are displayed in Supplementary Table S2. Of the validated miR-155 target genes, approximately half (48%) were predicted by TargetsScan software (23, 44). This highlights the drawbacks in relying on *in silico* prediction tools to investigate potential miRNA targets. The discrepancy between predicted and observed miR-155 binding sites is affected by miR-155 targeting nonconserved sites in target genes, as TargetsScan by default searches for seed sequences conserved between human and mouse. Carrying out a TargetsScan search irrespective of site conservation predicts 2,390 potential miR-155 targets and encompasses all but 9 validated target genes. This is the first comprehensive collation of all known miR-155 target genes and will be a valuable resource for future reference and research.

Although only a fraction of validated miR-155 target genes have a confirmed role in breast cancer, a number of the targets are involved in cancer-related pathways such as apoptosis, proliferation and EMT (20, 21, 32, 88, 89), as shown in Fig. 2. The presence of validated miR-155 targets in these pathways highlight the importance of miR-155 in cancer progression.

### Conclusion

As an oncomiR, expression levels of miR-155 are consistently upregulated in breast tumor samples. Studies have defined the clinical significance of miR-155 in breast cancer with an association with clinical markers, more aggressive tumors, and decreased survival. However, there are some contradictory findings reported, for instance, the varied association of miR-155 with hormone receptor status. It is also unclear as to whether miR-155 functions initiate cancer or predominantly promotes tumor progression. In a mouse model, miR-155 has been shown to transform B cells (90) but in breast cells has only been shown to enhance cancerous properties of tumor cells. More investigation is required to fully understand the significance of aberrantly high levels of miR-155 in breast cancer.

Exploration of the function of miR-155 in breast cancer cell lines and xenograft models shows that miR-155 enhances tumor growth, promotes cell proliferation, inhibits apoptosis, and acts as a mediator of TGF $\beta$ -driven EMT. In particular, the role of miR-155 in EMT has promising therapeutic potential, given that miR-155 levels have been shown to be elevated in invasive tumors and in breast tumor metastases. The large number of validated miR-155 targets presented in Supplementary Table S1 provide many avenues of further investigation as to the clinical potential of miR-155. The further investigation of these targets will be required to confirm the mechanistic



**Figure 2.** miR-155 target genes involved in cancer-related pathways. Validated miR-155 target genes are present in multiple pathways associated with cancer and cancer progression, including but not limited to EMT, proliferation, block of differentiation, apoptosis, sustained angiogenesis. Pathway analysis was completed using DAVID bioinformatics resource (v 6.7).

and regulatory actions of miR-155 and their contribution to breast cancer.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** S. Mattiske, R.J. Suetani

**Development of methodology:** S. Mattiske

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** S. Mattiske

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** S. Mattiske, R.J. Suetani

**Writing, review, and/or revision of the manuscript:** S. Mattiske, R.J. Suetani, P.M. Neilsen, D.F. Callen

**Study supervision:** R.J. Suetani, P.M. Neilsen, D.F. Callen

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