Breast Cancer Res Treat (2010) 121:227–231
DOI 10.1007/s10549-009-0509-8

BRIEF REPORT

# Frequent loss of heterozygosity at the interferon regulatory factor-1 gene locus in breast cancer

**Luciane R. Cavalli · Rebecca B. Riggins · Antai Wang · Robert Clarke · Bassem R. Haddad**

Received: 6 August 2009 / Accepted: 8 August 2009 / Published online: 21 August 2009


**Abstract** The interferon regulatory factor-1 (*IRF1*) gene, localized on chromosome 5q31.1, is mutated or rearranged in several cancers including some hematopoietic and gastric cancers. However, whether loss of *IRF1* occurs in sporadic breast cancer is unknown. Loss of 5q12-31 is reported in 11% of sporadic breast cancers, and high-resolution array-CGH studies have shown loss at 5q31.1 in 50% of breast cancers with a mutated *BRCA1* gene. Functionally, overexpression of *IRF1* reduces, and a dominant negative *IRF1* construct increases, tumorigenesis of human breast cancer xenografts. Taken together, these observations indicate that the *IRF1* gene may play a potentially important role as a breast cancer tumor suppressor gene. In this study, we investigated allelic loss of the *IRF1* gene in breast tumor specimens from 52 women with invasive breast cancer using an *IRF1* intragenic dinucleotide polymorphic marker. Thirty-seven cases were informative. LOH at the *IRF1* locus was detected in 32% of these informative cases (12/37). There was a significant association between *IRF1* loss and both older age ($P = 0.0167$) and earlier stage (Stages 1 and 2) ($P = 0.0165$). To assess the association of IRF1 mRNA expression with clinical outcomes in breast cancer, we studied data from two published gene expression microarray datasets. In breast cancer patients, low IRF1 mRNA expression is strongly correlated with both risk of recurrence (OR = 3.00; $P = 0.003$; $n = 273$ cases) and risk of death (OR = 4.18; $P = 0.004$; $n = 191$ cases). Our findings strongly imply a tumor suppressor role for the *IRF1* gene in breast cancer.

**Keywords** Interferon regulatory factor-1 · *IRF1* · Breast cancer · Disease survival · Tumor suppressor gene

## Introduction

The interferon regulatory factor-1 (*IRF1*) gene mediates interferon and other cytokine effects and exhibits antitumor activity in vivo and in vitro. *IRF1* can also reverse the oncogenic transformation of cells induced by the overexpression of both *RAS* and *MYC* in mouse models [1]. Since functional roles for *RAS* and *MYC* are established in human breast cancer [2, 3], a loss of *IRF1* function might be important in this disease. Functionally, overexpression of *IRF1* reduces [4, 5], and a dominant negative *IRF1* construct increases [4], tumorigenesis of human breast cancer xenografts. We and others have identified *IRF1* as a key regulator of breast cancer cell survival [5–8] that can activate a caspase cascade [4] and induce apoptosis [5, 6]. More specifically, the proapoptotic effects of *IRF1* include activation/regulation of caspases-1 [9], -3 [4], -7 [4, 10], and -8 [4, 11]. *IRF1* can also induce apoptosis through both

L. R. Cavalli · R. B. Riggins · R. Clarke · B. R. Haddad
Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, DC 20057, USA

A. Wang
Department of Biostatistics and Bioinformatics, Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, DC 20057, USA

B. R. Haddad
Department of Obstetrics and Gynecology, Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, DC 20057, USA

R. Clarke (✉)
Department of Oncology, Georgetown University School of Medicine, Room W405A Research Building, 3970 Reservoir Rd, NW, Washington, DC 20007, USA
e-mail: clarker@georgetown.edu

*TP53*-dependent and *TP53*-independent signaling [9, 12]. *TP53* is often mutated in breast cancer [13], and many breast tumors initially respond to drugs and hormones through *TP53*-dependent and *TP53*-independent signaling. We have shown that *IRF1* is a key determinant of responsiveness to antiestrogen therapies in breast cancer [6, 7].

Whether *IRF1* is a true tumor suppressor gene (TSG) in breast cancer is unknown. Established TSGs often show evidence of homozygous or heterozygous gene loss. For instance, while loss of *BRCA1* function in inherited breast cancers is usually a consequence of gene mutation(s), loss of *BRCA1* expression in sporadic breast cancers is often the result of loss of heterozygosity (LOH) accompanied by hypermethylation of a CpG island in the 5′ region close to the transcription start site of the remaining allele [14, 15]. *IRF1* has been implicated as a putative TSG in leukemias and preleukemic myelodysplasias, and *IRF1* is either mutated or rearranged in some hematopoietic disorders [16]. *IRF1* was shown to be the true target of frequent deletions (LOH) in esophageal cancer [17] and gastric cancer [18]. *IRF1* is located at 5q31.1, a region shown to be commonly lost in two large studies evaluating breast tumors by chromosomal comparative genomic hybridization (CGH). Deletion of 5q12-31 is detected in 11% of sporadic breast cancers [19] and 5q deletion is seen in 86% of *BRCA1* tumors [20]. More recently, a high-resolution array-CGH study has shown loss at 5q31.1 in 50% of *BRCA1*-positive breast cancers [21]. Whether loss at the *IRF1* locus is the driver in these cancers and whether *IRF1* gene loss occurs in sporadic breast cancers are unknown.

*IRF1* is inactivated by a point mutation in gastric cancer, suggesting that the loss of function of *IRF1* may be critical for the development of this disease [18]. When attempting to generate an *IRF1* riboprobe from MCF-7 breast cancer cells mRNA, we found a single nucleotide polymorphism (SNP) in the *IRF1* coding region [22] and a novel *IRF1* splice variant (K. B. Bouker; unpublished observations). The *IRF1* A4396G SNP is more frequent in human breast cancer cell lines than in the general population and is more frequently expressed in African-American than Caucasian subjects [22]. It is not known whether *IRF1* A4396G contributes to the earlier age [23] or higher stage at diagnosis [24] or the lower overall survival rate of African-American compared with non-Hispanic white and Hispanic women [25]. When considered together, these observations strongly suggest that *IRF1* may be a TSG in breast cancer. Since one of the key features of TSGs is somatic loss, we designed this study to determine the incidence of *IRF1* loss in a series of 52 invasive breast tumors. Considering that *IRF1* LOH might be expected to reduce mRNA expression, we also explored whether low *IRF1* mRNA expression is associated with poor clinical outcomes in breast cancer patients.

## Methods

We determined *IRF1* loss by LOH in breast tissue specimens from 52 patients with sporadic breast cancer obtained from the tumor bank at the Lombardi Comprehensive Cancer Center (LCCC). In each case, a paraffin block with breast tumor tissues and a second block with normal tissues (skin, negative lymph node, or a normal breast tissue block) were identified. An H&E-stained slide from each block was evaluated by a breast pathologist to confirm the diagnosis and mark the areas with malignant tissue or normal tissue. A 100-μm consecutive section was obtained from each block, and the tissues of interest were grossly microdissected with a razor blade to isolate malignant cells. Corresponding normal cells from a different block were obtained for each case. DNA was extracted from the tissue using the DNeasy QIAGEN kit according to manufacturer's instructions (QIAGEN Inc. Valencia, California).

To study LOH at the *IRF1* locus, we selected an intragenic, dinucleotide, polymorphic marker (*IRF1* Dinucleotide Repeat, Allele Set GDB: 211036), with a high degree of heterozygosity (74% heterozygosity). The sequences of the oligonucleotide primers were obtained from the Genome Data Base (GDB) (http://www.gdb.org): Forward: 5′-ATG GCAGATAGGTCCACCGG-3′/Reverse: 5′-TCATCCTCA TCTGTTGTACG-3′. Primers were fluorescently labeled and PCR amplification was performed using a standard protocol. Allele sizes were determined by electrophoresis of PCR products in 6% denaturing polyacrylamide gels and were compared to ROX 500 size standards (Applied Biosystems, Foster City, CA), using an automated sequencer (ABI 377), according to the manufacturer's instructions (Applied Biosystems). Fluorescent signals from the different size alleles were recorded and analyzed using GENOTYPER version 2.1 and GENESCAN version 3.1 software (Applied Biosystems). Following visual examination of computer printouts by two independent observers, LOH was determined mathematically according to the Genotyper User's Manual (Applied Biosystems).

The publicly available ONCOMINE cancer gene expression microarray database [www.oncomine.org; 26] was used to search for relationships between *IRF1* mRNA expression and outcomes in breast cancer clinical studies. Normalized Affymetrix MAS 5.0 gene expression data, originally published by Wang et al. [27] and Desmedt et al. [28], were downloaded from ONCOMINE. Statistical analysis was done using SigmaStat (Systat Software, Inc., San Jose, CA) and S-PLUS (Insightful, Seattle, WA). Median *IRF1* expression values across all samples, and between the top and bottom quartiles of expression, were compared by Mann–Whitney Rank-Sum test, and odds ratios were calculated for the association of low *IRF1* expression with poor outcomes following logistic

regression analysis. Statistical significance was defined as ≥95% confidence interval, or $\alpha = 0.05$.

## Results

In this study, 37 cases (71%) were informative for the *IRF1* dinucleotide marker used for LOH analysis. LOH was detected in 12 of these informative cases (12/37; 32%). Figure 1 shows a representative case with no LOH (Fig. 1a, bottom panel) and another representative case with LOH (Fig. 1b, bottom panel). A significant correlation was found between LOH at the *IRF1* locus and both older age ($P$ value = 0.0167 based on a two sample $t$-test) and earlier stage (Stages 1 and 2) ($P$ value = 0.0165 based on Fisher's exact test).

The data from two published gene expression microarray datasets were assessed to investigate mRNA expression and clinical outcome [27, 28]. In both studies, median *IRF1* mRNA expression, as detected by Affymetrix U133A GeneChips, was significantly reduced in patients with a worse outcome (recurrence, $P = 0.004$; death, $P = 0.021$). Moreover, when these expression values were separated into quartiles and analyzed by logistic regression (Table 1), low *IRF1* mRNA expression (first compared with fourth quartile) was significantly associated with both recurrence (OR = 3.00, $P = 0.003$) and death (OR = 4.18, $P = 0.004$).

## Discussion

In our study, a significant correlation was found between LOH at the *IRF1* locus and both older age ($P = 0.0167$) and earlier stage (Stages 1 and 2) ($P = 0.0165$). An inverse association between IRF1 protein expression and tumor grade has been reported [29], and IRF1 protein levels are lower in breast tumors than in adjacent normal cells [30]. However, subcellular location also affects IRF1 correlation with clinical measures, and these correlations would not be apparent with LOH measurements. For example, cytosolic IRF-1 protein (but not nuclear *IRF1*) is associated with age (similar to LOH findings) and ER expression, whereas nuclear IRF1 protein expression (but not cytosolic *IRF1*) correlates with PgR expression [31].

Since *IRF1* LOH might be expected to reduce mRNA expression, we explored the association of *IRF1* mRNA with the key measures of recurrence status (recurrent; non-recurrent) and vital status (alive; dead). Using the publicly available ONCOMINE cancer gene expression microarray database [26], we searched for relationships between *IRF1* mRNA expression and outcomes from two breast cancer clinical studies. The Wang et al. study includes 273 women diagnosed with lymph node-negative breast cancer who had not received systemic adjuvant therapy, but whose tumors are representative of a wide range of clinical/pathological features (including age, stage, and tumor size). The original goal of that study was to identify a gene expression signature that could predict recurrence of metastatic breast cancer in women with node-negative disease [27]. The more recent Desmedt et al. study is an independent validation of Wang et al. and includes 191 untreated patients less than 61 years of age with node-negative, T1 and T2 breast cancer [28]. The primary endpoint for the study was overall survival, and median follow-up time was 13.6 years. While *IRF1* gene copy number data and protein expression data were not available from these studies, we could assess the potential of *IRF1* to act as a TSG as predicted by the likelihood that low *IRF1* mRNA expression was associated with poor clinical outcomes. We have observed that low IRF1 mRNA expression is strongly correlated with both risk of recurrence and risk of death. Studies to determine directly the role of *IRF1* LOH in these outcomes are in progress.

*IRF1* LOH in breast cancer may reflect haploinsufficiency; over 35 TSGs are known in which haploinsufficiency accounts for their contribution to carcinogenesis (though not necessarily breast carcinogenesis) [32, 33]. Reduced IRF1 activity in experimental breast cancer models is functionally associated with increased cell survival,

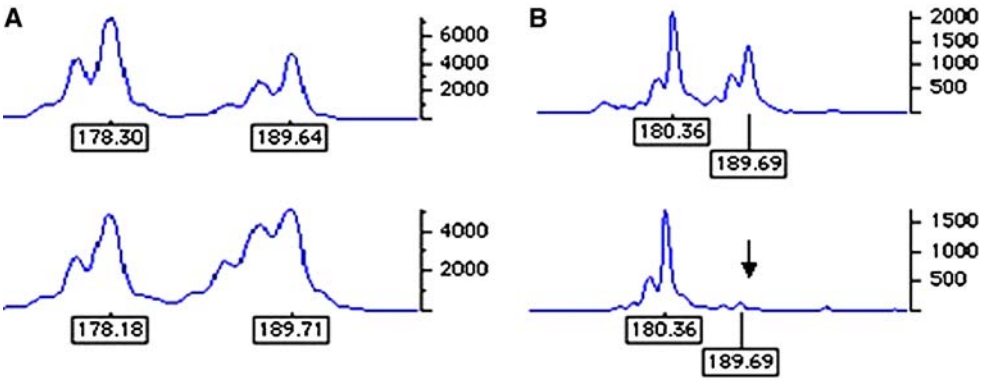


**Fig. 1** LOH analysis at the *IRF1* locus of two representative breast cancer cases. **a** A case with no LOH. **b** A case with LOH. (In **a**, **b**, *Top panels* shows the analysis performed in normal cells and *bottom panels* the analysis performed in tumor cells.)

**Table 1** *IRF1* mRNA expression and clinical outcomes

| Study | Endpoint | *IRF1* (median) | *IRF1* (quartile) | Odds ratio[a] |
|---|---|---|---|---|
| Wang et al. | No disease ($n = 180$) | $P = 0.004$ | First = 0.971 | OR = 3.00 |
| | | | | CI = 1.44–6.27 |
| | Recurrence ($n = 93$) | | Fourth = 0.455 | $P = 0.003$ |
| Desmedt et al. | Alive ($n = 135$) | $P = 0.021$ | First = 0.905 | OR = 4.18 |
| | | | | CI = 1.56–11.21 |
| | Dead ($n = 56$) | | Fourth = 0.355 | $P = 0.004$ |

[a] Odds ratios were calculated for the first (highest) versus fourth (lowest) quartiles of expression data, and denote the association of low *IRF1* mRNA expression with poor outcome (recurrence or death); CI = 95% confidence interval

reduced caspase activation, and apoptosis (4–6). While LOH may not be the only contributor to low *IRF1* expression [34, 35], the strong association of low *IRF1* mRNA and recurrence status and survival imply an important TSG role for *IRF1* in many sporadic breast cancers.

**Acknowledgments** This study was supported in part by a pilot grant from the Jess and Mildred Fisher Center for Familial Cancer Research at Georgetown University, BC030280 from the DOD, and KG091175 from the Komen Foundation. We thank the Histopathology and Tissue Shared Resource (HTSR) of the Lombardi Comprehensive Cancer Center, which is partially supported by National Institutes of Health Grant 1P30-CA-51008 (Cancer Center Support Grant, CCSG), for providing the histopathology support.

## References

1. Nozawa H, Oda E, Nakao K, Ishihara M, Ueda S, Yokochi T, Ogasawara K, Nakatsuru Y, Hioki K, Aizawa S, Ishikawa T, Katsuki M et al (1999) Loss of transcription factor IRF-1 affects tumor susceptibility in mice carrying the Ha-ras transgene or nullizygosity for p53. Genes Dev 1–3:1240–1245
2. Bos JL (1989) Ras oncogenes in human cancer: a review. Cancer Res 49:4682–4689
3. Deming SL, Nass SJ, Dickson RB, Trock BJ (2000) C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. Br J Cancer 83:1688–1695
4. Bouker KB, Skaar TC, Riggins R, Harburger DS, Fernandez DR, Zwart A, Wang A, Clarke R (2005) Interferon regulatory factor-1 (IRF-1) exhibits tumor suppressor activities in breast cancer associated with caspase activation and induction of apoptosis. Carcinogenesis 26:1527–1535
5. Kim PKM, Armstrong M, Liu Y, Yan P, Bucher B, Zuckerbraun BS, Gambotto A, Billiar TR, Yim JH (2004) IRF-1 expression induces apoptosis and inhibits tumor growth in mouse mammary cancer cells in vitro and in vivo. Oncogene 23:1125–1135
6. Gu Z, Lee RY, Skaar TC, Bouker KB, Welch JN, Lu J, Liu A, Zhu Y, Davis N, Leonessa F, Brunner N, Wang Y et al (2002) Association of interferon regulatory factor-1, nucleophosmin, nuclear factor-kappaB, and cyclic AMP response element binding with acquired resistance to faslodex (ICI 182, 780). Cancer Res 62:3428–3437
7. Bouker KB, Skaar TC, Fernandez DR, O'Brien KA, Clarke R (2004) Interferon regulatory factor-1 mediates the proapoptotic but not cell cycle arrest effects of the steroidal antiestrogen ICI 182, 780 (Faslodex, Fulvestrant). Cancer Res 64:4030–4039
8. Bowie ML, Dietze EC, Delrow J, Bean GR, Troch MM, Marjoram RJ, Seewaldt VL (2004) Interferon-regulatory factor-1 is critical for tamoxifen-mediated apoptosis in human mammary epithelial cells. Oncogene 23:8743–8755
9. Tamura T, Ishihara M, Lamphier MS, Tanaka N, Oishi I, Alzawa S, Matsuyama T, Mak TW, Taki S, Taniguchi T (1995) An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. Nature 376:596–599
10. Sanceau J, Hiscott J, Delattre O, Wietzerbin J (2000) IFN-beta induces serine phosphorylation of stat-1 in Ewing's sarcoma cells and mediates apoptosis via induction of IRF-1 and activation of caspase-7. Oncogene 19:3372–3383
11. Suk K, Chang I, Kim YH, Kim S, Kim JY, Kim H, Lee MS (2001) Interferon gamma (IFNgamma) and tumor necrosis factor alpha synergism in ME-180 cervical cancer cell apoptosis and necrosis. IFNgamma inhibits cytoprotective NF-kappa B through STAT1/IRF-1 pathways. J Biol Chem 276:13153–13159
12. Tanaka N, Ishihara M, Lamphier MS, Nozawa H, Matsuyama T, Mak TW, Aizawa S, Tokino T, Oren M, Taniguchi T (1996) Cooperation of the tumour suppressors IRF-1 and p53 in response to DNA damage. Nature 382:816–818
13. Elledge RM, Allred DC (1994) The p53 tumor suppressor gene in breast cancer. Breast Cancer Res Treat 32:39–47
14. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M et al (2000) Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92:564–569
15. Wei M, Grushko TA, Dignam J, Hagos F, Nanda R, Sveen L, Xu J, Fackenthal J, Tretiakova M, Das S, Olopade OI (2005) BRCA1 promoter methylation in sporadic breast cancer is associated with reduced BRCA1 copy number and chromosome 17 aneusomy. Cancer Res 65:10692–10699
16. Willman CL, Sever CE, Pallavicini MG, Harada H, Tanaka N, Slovak ML, Yamamoto H, Harada K, Meeker TC, List AF, Taniguchi T (1993) Deletion of IRF-1, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia. Science 259:968–971
17. Ogasawara S, Tamura G, Maesawa C, Suzuki Y, Ishida K, Satoh N, Uesugi N, Saito K, Satodate R (1996) Common deleted region on the long arm of chromosome 5 in esophageal carcinoma. Gastroenterology 110:52–57
18. Nozawa H, Oda E, Ueda S, Tamura G, Maesawa C, Muto T, Taniguchi T, Tanaka N (1998) Functionally inactivating point mutation in the tumor-suppressor IRF-1 gene identified in human gastric cancer. Int J Cancer 77:522–527
19. Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J, Kallioniemi OP (1998) Molecular cytogenetics of primary breast cancer by CGH. Genes Chromosomes Cancer 21:177–184

20. Tirkkonen M, Johannsson O, Agnarsson BA, Olsson H, Ingvarsson S, Karhu R, Tanner M, Isola J, Barkardottir RB, Borg A, Kallioniemi OP (1997) Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 57:1222–1227
21. Johannsdottir HK, Jonsson G, Johannesdottir G, Agnarsson BA, Eerola H, Arason A, Heikkila P, Egilsson V, Olsson H, Johannsson OT, Nevanlinna H, Borg A et al (2006) Chromosome 5 imbalance mapping in breast tumors from BRCA1 and BRCA2 mutation carriers and sporadic breast tumors. Int J Cancer 119: 1052–1060
22. Bouker KB, Skaar TC, Harburger DS, Riggins R, Fernandez DR, Zwart A, Clarke R (2007) The A4396G polymorphism in interferon regulatory factor-1 is frequently expressed in breast cancer. Cancer Genet Cytogenet 175:61–64
23. Aziz H, Hussain F, Sohn C, Mediavillo R, Saitta A, Hussain A, Brandys M, Homel P, Rotman M (1999) Early onset of breast carcinoma in African American women with poor prognostic factors. Am J Clin Oncol 22:436–440
24. Weir HK, Thun MJ, Hankey BF, Ries LA, Howe HL, Wingo PA, Jemal A, Ward E, Anderson RN, Edwards BK (2003) Annual report to the nation on the status of cancer, 1975–2000, featuring the uses of surveillance data for cancer prevention and control. J Natl Cancer Inst 95:1276–1299
25. Shavers VL, Harlan LC, Stevens JL (2003) Racial/ethnic variation in clinical presentation, treatment, and survival among breast cancer patients under age 35. Cancer 97:134–147
26. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A, Chinnaiyan AM (2004) ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 6:1–6
27. Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM et al (2005) Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365:671–679
28. Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, Viale G, Delorenzi M, Zhang Y, d'Assignies MS, Bergh J, Lidereau R et al (2007) Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. Clin Cancer Res 13:3207–3214
29. Connett JM, Badri L, Giordano TJ, Connett WC, Doherty GM (2005) Interferon regulatory factor 1 (IRF-1) and IRF-2 expression in breast cancer tissue microarrays. J Interferon Cytokine Res 25:587–594
30. Doherty GM, Boucher L, Sorenson K, Lowney J (2001) Interferon regulatory factor expression in human breast cancer. Ann Surg 233:623–629
31. Zhu Y, Singh B, Hewitt S, Liu A, Gomez B, Wang A, Clarke R (2006) Expression patterns among interferon regulatory factor-1, human X-box binding protein-1, nuclear factor kappa B, nucleophosmin, estrogen receptor alpha and progesterone receptor proteins in breast cancer tissue microarrays. Int J Oncol 28:67–76
32. Payne SR, Kemp CJ (2005) Tumor suppressor genetics. Carcinogenesis 26:2031–2045
33. Santarosa M, Ashworth A (2004) Haploinsufficiency for tumour suppressor genes: when you don't need to go all the way. Biochim Biophys Acta 1654:105–122
34. Book MM, Yu-Lee LY (2001) Sp1 is required for prolactin activation of the interferon regulatory factor-1 gene. Mol Cell Endocrinol 184:135–141
35. Li X, Leung S, Qureshi S, Darnell JE Jr, Stark GR (1996) Formation of STAT1-STAT2 heterodimers and their role in the activation of IRF-1 gene transcription by interferon-alpha. J Biol Chem 271:5790–5794