Molecular Cloning and Characterization of a Zinc Finger Protein Involved in Id-1-stimulated Mammary Epithelial Cell Growth*

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Id proteins are dominant negative regulators of basic helix-loop-helix transcription factors. Previous work in our laboratory has shown that constitutive expression of Id-1 in SCp2 mouse mammary epithelial cells inhibits their differentiation and induces proliferation, invasion, and migration. Id-1 expression also correlates with the invasive and aggressive potential of human breast cancer cells. However, little is known about Id-1 target genes that are important for regulating normal and transformed breast epithelial cell phenotypes. Now we report the cloning of a novel zinc finger protein, Zfp289, using degenerate primers to specifically amplify cDNAs from Id-1-transfected SCp2 cells. Zfp289 has homology with a yeast zinc finger protein, the GTPase-activating protein Ges-1, which was initially identified as a gene required for the re-entry of cells into the cell cycle after stationary phase growth. Zfp289 mRNA expression pattern correlates with Id-1 expression in SCp2 mammary epithelial cells under various experimental conditions as well as in the mouse mammary gland at different stages of development. It is predominantly present in the cytoplasm of the cells as evident from green fluorescent protein fusion protein localization. SCp2 mammary epithelial cells with constitutive expression of Zfp289 have a higher S-phase index, compared with control cells, when cultured in a serum-free medium. We conclude that the novel zinc finger protein Zfp289, which may represent the mammalian homologue of Ges-1, is potentially an important mediator of the Id-1-induced proliferation pathway in mammary epithelial cells.

Basic helix-loop-helix (bHLH) factors are transcription factors that bind DNA as homo- or heterodimers and regulate transcription of target genes containing E-boxes (CANNTG) or E-box-like sequences in their promoters. Dimerization occurs through interactions of the HLH domains, while binding to DNA is mediated by the basic domain. These factors have been shown to regulate the expression of tissue-specific genes in a number of mammalian and nonmammalian organisms (1). Id proteins (for “inhibitors of differentiation or DNA binding”) are dominant negative regulators of the bHLH transcription factors. Id proteins contain an HLH domain, allowing them to form dimers with bHLH proteins, but they lack the basic domain, and therefore such dimers, Id/bHLH, do not bind DNA (2). Therefore, Id proteins do not regulate transcription directly, but indirectly, by preventing bHLH proteins from interacting with the promoter of various target genes. The role of Id proteins in the tissue-specific regulation of growth and differentiation has been examined in several systems. For example, Id-1 has been found to inhibit differentiation in myoblast (2), trophoblast (3), erythroid (4), B-lymphocyte (5, 6), and myeloid cells (7).

Previous studies in our laboratory have shown that constitutive expression of Id-1 results in the inhibition of differentiation of SCp2 mouse mammary epithelial cells (8). It also induces proliferation, invasion, and migration of the same cells (9) and increased secretion of a 120-kDa matrix metalloproteinase, the level of which correlates well with the invasive ability of these cells. In addition, Id-1 is highly expressed in aggressive and invasive human breast cancer cell lines as compared with noninvasive cell lines (9) and in biopsies from invasive ductal carcinomas as compared with ductal carcinomas in situ (10). Investigations have shown that Id-1 is a positive regulator of G1-S phase transition during cell cycle progression and is also involved in inducing apoptosis (11–16). A recent report demonstrated that Id-1 and Id-3 might also control angiogenesis by regulating the growth and invasion of endothelial cells (17). However, little is known about Id target genes, which are important for regulating growth, differentiation, invasion, and apoptosis of normal and transformed mammary epithelial cells.

In this paper, we report the cloning of a novel Id-1-induced zinc finger protein, Zfp289, which is predominantly localized in the perinuclear compartment of the cells and which appears to function as a GTPase-activating protein (GAP). Zfp289 expression is correlated with the proliferative stages of mammary epithelial cells in culture and during mammary gland development, and this novel zinc finger protein is able to induce higher S-phase entrance when constitutively expressed in epithelial cells.

MATERIALS AND METHODS

cDNA Cloning of Zfp289—We used PCR amplification to isolate genes specifically regulated by Id-1, as indicated by their up-regulation in SCp2 cells transfected with Id-1. Our rational for selecting the degenerate primers was that we previously demonstrated a novel matrix metalloproteinase family member to be up-regulated in SCp2 cells transfected with Id-1 (9). Since we were particularly interested in cloning this novel metalloproteinase, and since most of the known metalloproteinases have a “Cys” motif and a “zinc” binding motif in
their sequence, we designed degenerate primers against two regions of
terest, one containing a cysteine residue Cys (PRCGXPD), the other a
catalytic domain binding zinc ions (VAAHEFGHALGLH). Cys and zinc
sequences were as followed: 5′-Sc(e/g)GR(e/g) Sc(e/g/W
(a/t)Rg/a) CCN(a/e/g/t) GA-3′ and 5′-GCR(e/g) TG8(g/e) CCV(a/e/g)
AAY(o/t) TCR(g/a) TGS(e/g) GC-3′.

Total RNA was isolated from SCp2-Id-1-transfected cells and SCp2
control cells, and cDNA was prepared. Specific AP-1 adaptors (Marathon
cDNA amplification kit) were ligated at both ends of the cDNAs, and a first round of PCR amplification was performed using adaptor-
specific primers on one side and zinc primers on the other. A second
round of amplification was performed using the PCR products from
the first round and using Cys and zinc primers. Only two amplified prod-
ucts of 0.8 and 2.6 kb were visible. We used annealing temperatures of
45 and 50 °C to obtain sharp bands at 0.8 and 2.6 kb, respectively. Both
of these bands were extracted and cloned into a TOPO vector for sequenc-
ing.

Cell Culture—SCp2 mouse mammary epithelial cells were grown in a
1:1 mixture of Dulbecco’s modified Eagle’s medium and F-12 (Dulbe-
cco’s modified Eagle’s medium-F-12) containing 5% heat-inactivated feta-
tal bovine serum and insulin (5 µg/mL), at 37 °C in a humidified 5% CO2
atmosphere, as described previously (18). For experiments in serum-
starved conditions, fetal bovine serum was omitted. Pool populations of
SCp2 cells transfected with an empty vector or with the murine Id-1
sense cDNA driven by the mouse mammary tumor virus promoter were
as described previously (8). Single cell-derived clones from SCp2 cells
transfected with an Id-1 antisense cDNA (8) were derived by plating
cells at limiting dilutions in 24-well plates.

Plasmids Construction and Transfection—The Zfp289 encoding re-
ing including the Kozak sequence was amplified from the BamHI site
at the 5′-end, to either BamHI (for LXSN vector) or Sall site (for pBabe
vector) at the 3′-end. The restriction digested fragments were then
cloned into appropriate sites of LXSN and pBabe vectors. These viral
vectors were then packaged in TSA-54 cells (Cell Genesis, Foster City, CA). After infecting the SCp2 cells with control or Zfp289 vectors, stably
transfected cells were selected with neomycin and puromycin (for LXSN
and pBabe vectors, respectively).

For intracellular localization studies, the full-length coding sequence
of Zfp289 was cloned into a pEGFP vector (CLONTECH) between the
SalI and BamHI sites. The pEGFP vector was transfected using Super-
fect transfection reagent (Qiagen). Neomycin-resistant cells were sub-
cultured, and localization of the GFP fusion protein was determined
under inverted fluorescent microscopy.

RNA Isolation and Northern Analysis—Total cellular RNA was iso-
lated and purified as described by Chomczynski and Sacchi (19). RNA
(15 µg) was size-fractionated by electrophoresis through denaturing
formaldehyde-agarose gels and transferred to Nylon membrane (Hy-
bond-N from Amersham Pharmacia Biotech). The blots were hybridized
with 32P-labeled probes prepared by random oligonucleotide priming,
was prepared and exposed to Kodak XAR-5 film for autoradiography (20).
The multiple tissue Northern blot was purchased from CLONTECH
and probed as above. The Zfp289 probe was the PCR-amplified 2.6 kb
fragment described under “cDNA Cloning of Zfp289,” whereas β-casein
and Id-1 probes were as described previously (8). The probe was obtained
by subtractive hybridization, and the β-actin probe was obtained from CLONTECH.

Preparation of Mammary Gland RNA—BALB/c wild type virgin fe-
naline was purchase from Simonsen Laboratories, Inc. (Gilroy, CA)
and some were mated at the age of 12 weeks. The animals were
sacrificed, and biopsies of the fourth inguinal mammary gland were
performed at 3, 7, and 12 weeks of age for the virgin stage; at days 2 and
12 of pregnancy; at days 2, 7, 20, and 21 of lactation. Mammary
glands were immediately frozen at −70 °C until utilized for total RNA
isolation. Total RNA was isolated using TriPure Isolation reagent
(Roche Molecular Biochemicals).

DNA Synthesis and Autoradiography—Cells (104 or 5 × 104) plated
on coverslips were labeled with 3H[3H]methylthymidine (10 µCi/mL; 60–70
Ci/mm) for at least 7 h, washed twice with phosphate-buffered saline,
and then fixed for 5 min with 1:1(v/v) mixture of acetone and methanol
kept at −20 °C. Nuclei were stained with 4,6-diamidino-2-phenylindole-di-
dium hydrochloride, bacteria were pelleted

and lysed under denaturing conditions in buffer containing 6 M guani-
dine hydrochloride, 10 mM Tris-HCl, 100 mM Na2PO4, pH 8.0, for 1 h at

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room temperature with continuous shaking. Cellular debris was then pelleted by centrifugation of cell lysate at 10,000 × g for 30 min at room temperature. The supernatant was mixed with nickel-nitrilotriacetic acid beads (Qiagen) and stirred for 1 h at room temperature. The beads were then transferred to a column and sequentially washed with Buffer 1 (6 M urea, 20 mM Tris-HCl, 0.5 M NaCl, pH 8.0) and Buffer 2 (20 mM Tris-HCl, 0.15 M NaCl, pH 8.0) to remove the nonspecific proteins and to renature the nickel-nitrilotriacetic acid-bound proteins (21). The 6His-tagged ZFP-289 protein was eluted with Buffer 2 containing 50 mM EDTA. The eluted protein was concentrated by using centricon FIG. 2. Nucleotide sequence and deduced amino acid sequence of murine Zfp289. The N-terminal zinc finger domain is underlined, and the putative polyadenylation signal is shaded.

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Buffer 4 (8 M urea, 10 mM Tris-HCl, 0.1 M Na2PO4, pH 5.9) and then was renatured by sequential dialysis against 4 M urea, 100 mM Na2PO4, 10 mM Tris-HCl, pH 8.0, then against 2 M urea, 10 mM Tris-HCl, pH 8.0, then against 2 M urea, 10 mM Tris-HCl, pH 6.3). The bound [32P]GTP was then diluted 10 times with binding buffer (20 mM Tris-HCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 1 μg/ml bovine serum albumin, pH 7.5) and then again with Buffer 4 containing 250 mM imidazole. The eluted ARF-1 was renatured by sequential dialysis against 4 M urea, 100 mM Na2PO4, 10 mM Tris-HCl, pH 6.3, then against 2 M urea 10 mM Tris-HCl, pH 8.0, and finally twice against 10 mM Tris-HCl, pH 8.0. The renatured ARF-1 was concentrated, and its purity (about 80–85%) was determined by SDS-polyacrylamide gel electrophoresis.

ARF-GAP Assay—ARF-GAP activity was assayed by measuring the effects of the putative GTPase-activating protein (Zfp289) on the hydrolysis of ARF-bound GTP to GDP, with some modifications of the assay previously described by Goldberg (22). Briefly, ARF-1 was incubated with [γ-32P]GTP for 30 min in binding buffer (20 mM Tris-HCl, 0.1 mM Na2PO4, pH 5.9) and then again with Buffer 4 containing 250 mM imidazole. The eluted ARF-1 was reconstituted with ARF-1 and washed four times with Tris buffer saline, and radioactivity was assayed by scintillation counting. The potential GTPase-activating protein activity of Zfp289 was determined as the decrease of [γ-32P]GTP. ARF-1 ([γ-32P]GTP) was then diluted 10 times with binding buffer. GTase assay was done by incubating GTP-bound ARF-1 with or without Zfp289 at room temperature for 20 min in a 25-μl reaction mixture. After incubation, the samples were spotted on nitrocellulose membrane, washed four times with Tris buffer saline, and radioactivity was assayed by scintillation counting. The potential GTPase-activating protein activity of Zfp289 was determined as the decrease of label associated with ARF-1.

RESULTS

Isolation of Two Id-1-inducible Genes in SCp2 Cells—Using degenerate primers as described under "Materials and Methods," we isolated two cDNA clones (0.8 and 2.6 kb) preferentially up-regulated in SCp2-Id-1-transfected cells. A partial sequencing of the 0.8-kb band revealed that it corresponded to a known gene encoding histone H3.3. Since we only performed a partial sequencing of this clone and did not analyze it further, we could not ascertain the presence of the zinc and Cys motifs. Using this cDNA as a probe, two mRNAs of 1.2 and 1.8 kb were detected in SCp2 cells cultured in 5% serum (Fig. 1A, lane 1) and in SCp2-Id-1 serum-starved for 2 days (Fig. 1A, lane 3).

The other 2.6-kb band corresponded to a gene encoding a mRNA of about 2.9 kb. We found a higher expression of this 2.9-kb transcript in serum-starved Id-1-transfected cells (Fig. 1A, lane 3) as compared with control cells, which were also serum-starved for 2 days and which showed a reduced amount of Id-1 protein (Fig. 1A, lane 2).

We detected a high level of expression of this 2.9-kb transcript in control SCp2 cells cultured in the presence of 5% serum (Fig. 1A, lane 1). This level of expression was not further increased in Id-1-transfected SCp2 cells also cultured in 5% serum (data not shown). This may be due to the large amount of endogenous Id-1 proteins, up-regulated by serum in control cells, which may be sufficient to interact with all bHLH proteins present. The level of expression of target genes, such as the one encoding the 2.9-kb transcript, may then correspond to a maximum.

As a control of the loading, we used ethidium bromide staining of ribosomal RNA as well as β-actin, which did not show a difference of expression at the mRNA level between control and Id-1-transfected cells in serum-starved conditions for 2 days. However, mRNA levels of another gene, clusterin, a glycoprotein involved in cell-cell interaction, were down-regulated in the presence of Id-1.

To establish further that Id-1 up-regulates expression of Zfp289, we analyzed mRNA levels of both genes in nine different clones from SCp2 cells transfected with Id-1 antisense vectors (8) and treated with growth factors, lactogenic hormones, and extracellular matrix. As shown in Fig. 1B, these clones expressed variable amounts of Id-1. In each of these clones, the levels of Zfp289 strongly correlated with that of Id-1. Four clones (lanes 1, 4, 6, and 8) expressed high levels of Id-1 and Zfp289, whereas in five clones (lanes 2, 3, 5, 7, and 9) Id-1 expression was considerably reduced, and therefore Zfp289 mRNA levels were significantly down-regulated. As described previously (8, 9), the expression of β-casein (a differentiation-related gene) was inversely correlated with that of Id-1.

Sequence Analysis—The nucleotide sequence analysis of the 2.6-kb fragment (plus the sequence of overlapping expressed sequence tags at the 5′- and 3′-ends) revealed an open reading frame of 1560 base pairs, encoding a 519-amino acid polypeptide with a predicted molecular mass of about 57 kDa (Fig. 2). We could clearly detect a sequence on this cDNA that was homologous to the zinc degenerate primer. We attempted to determine any sequence homology to the Cys motif, but due to the level of degeneracy of this Cys primer, we were not able to locate any region of clear homology. A search of the protein database revealed that this predicted 57-kDa protein was a unique sequence, having, however, a 48% homology and 32% identity with the yeast zinc finger protein Gcs-1 (23). This putative mouse protein, which we called Zfp289, after submission to the nomenclature committee, has one zinc finger domain at the N-terminus, with a CxxCxxC motif encompassing 26–49 amino
This zinc finger domain has 66% homology with the zinc finger domain in the yeast protein Gcs-1.

Tissue Distribution—
Northern blot analysis of Zfp289 revealed a predominantly 2.9-kb transcript in all the major murine tissues (Fig. 3), although the level of expression varied widely. Zfp289 mRNA was expressed at very high levels in liver, followed by heart and kidney. Skeletal muscle and spleen had the lowest levels of mRNA expression.

During mammary gland development in mice, Zfp289 mRNA expression pattern was closely correlated with Id-1 expression (Fig. 4). We detected high levels of expression of Zfp289 as well as Id-1 in the mammary gland from virgin (V) mice and during pregnancy (P) when there is extensive ductal cell proliferation and lobulo-alveolar development, respectively. The expression of both genes declined at the beginning of lactation (L) when the glands fully differentiate and express the milk product β-casein. Zfp289 and Id-1 were almost undetectable after day 2 of lactation until day 21.

Cellular Localization—
Localization studies using EGFP vector-transfected SCp2 cells showed that GFP-Zfp289 fusion protein was predominantly present in the cytoplasm with a high proportion of cells showing perinuclear staining (Fig. 5, B, C, and D). These data suggest that Zfp289 is not a transcription factor, which is corroborated by the fact that it contains only one zinc finger domain and not several like typical transcription factors belonging to the zinc finger protein family. This also suggests a function in the regulation of exocytic and/or endocytic vesicular transport pathways at the periphery of the nuclei.

Functional Analysis of Zfp289—To investigate the functional role of Zfp289, we stably transfected the SCp2 mouse mammary epithelial cells with two different mammalian expression vectors (LXSNS and pBabe) containing the full-length coding region of Zfp289. Northern blot analysis of total RNA from these cells confirmed the constitutive transcription of

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**Fig. 5. Cellular localization of Zfp289.** SCp2 cells were transfected with an empty GFP plasmid (A) or with a GFP plasmid containing the Zfp289 coding region (B, C, and D) and analyzed using fluorescent microscope.

**Fig. 6. A,** Northern blot analysis showing expression of Zfp289 transgene in SCp2 mammary epithelial cells. B, thymidine incorporation in SCp2 cells transfected with either control plasmid (LXSNS or pBabe) or plasmid containing full-length coding region of Zfp289 gene. Data represent the average of four independent experiments and is presented as percentage of control. One-way ANOVA comparing Zfp289-transfected cells with control cells was significantly different at p < 0.001.

**Fig. 7.** Analysis of the GTPase activity of ARF-1 in the presence of two different amounts of recombinant Zfp289 protein after 20-min incubation. The data shown are from one of three independent experiments which showed similar differences. One-way ANOVA comparing lane 4 versus lane 1 was not statistically different (p = 0.074), whereas one-way ANOVA comparing lane 2 (or lane 3) versus lane 4 was statistically different at p < 0.0001.
transfected Zfp289, which displayed a larger size than the
endogenous Zfp289 mRNA (Fig. 6A). In low serum conditions,
constitutive expression of Zfp289 resulted in higher S-phase
rate of mammary epithelial-transfected SCp2 cells as com-
pared with control plasmid-transfected cells (Fig. 6B).
The difference was more significant in the case of cells transfected
with LXSN (170% of the control) than with pBabe (150% of
the control). This may be due to a higher level of the transgene
in the LXSN vector than in the pBabe vector.

Zfp289 does not appear to play a role in the invasive behavior
of the cells. Even after 2 weeks on extracellular matrix, both
cell control as well as Zfp289-transfected cells remained associated
within compact spheres (data not shown). In contrast, consti-
tutive expression of Id-1 was able to induce invasion in SCp2
cells (9). We conclude that Zfp289 may be a downstream gene
under the control of the transcriptional regulators of the helix-
loop-helix family. This novel zinc finger protein is potentially
an important mediator of the Id-1-induced proliferation path-
way, but not of Id-1-induced invasiveness and/or migration, in
mammary epithelial cells.

Zfp289 Appears to Be a GAP Protein—We noted above the
homology of sequence between Zfp289 and the yeast GAP
Gcs-1. Since Zfp289 also contains the zinc finger motif shared
by most of the GAP proteins, we examined whether Zfp289 was
functionally a GTPase-activating protein. We analyzed the
GTPase activity of ARF-1 in the presence of two different
amounts of recombinant Zfp289 protein. ARF (ADP-ribo-
sylation) protein is 20-kDa guanine nucleotide-binding
proteins that are active when GTP is bound. However, hydrol-
ysis of bound GTP requires interaction with a GAP protein, as
ARF itself has no detectable GTPase activity. Zfp289 displays
a strong GAP activity as demonstrated by the decrease of label
associated with ARF-1 (Fig. 7). This is particularly significant
at 200 ng of Zfp289 and already detectable at 50 ng. Zfp289
may therefore correspond to the mammalian counterpart of the
yeast GAP protein, Gcs-1.

DISCUSSION

We have previously reported that Id-1, a dominant negative
regulator of bHLH transcription factors, is not only involved in
the inhibition of differentiation, but also induces proliferation,
migration, and invasion in SCp2 mouse mammary epithelial
cells (8, 9). To learn more about its action, we sought here to
identify transcripts up-regulated in cells transfected with Id-1.
Of the two transcripts we identified, one was the histone H3.3.
This is consistent with the up-regulation of H3.3 during the G0
or S-phase transition in mouse kidney cells (24) and suggests
that histone H3.3 is one of the mediators of Id-1-controlled
proliferation.

Of more interest was the second up-regulated transcript,
Zfp289, which encodes for a zinc finger protein with homology
to the yeast zinc finger protein Gcs-1 and its related protein Glo-3.
Gcs-1 was first identified because of its requirement for transi-
tion of yeast cells from stationary to proliferation phase (23).
Reports have also shown that Gcs-1 protein is a GAP for the ARF
(25) and is involved in regulation of vesicular trafficking and
actin cytoskeleton network (26). Yeast cells containing a func-
tionally mutant Gcs-1 gene were unable to transit from the
stationary phase to growth phase (23) and exhibited vesicle traf-
ficking defects at the nonpermissive 15 °C temperature (27).
Zfp289 seems to be evolutionarily a well conserved protein,
with about 50% homology between mammalian and yeast se-
quences, suggesting a conservation of function as well. The
GAP activity of Gcs-1 has been localized in the N-terminal zinc
finger domain (28), and the Zfp289 sequence in this finger
domain (CxxCxx(16)CxxC) is 66% homologous to that of yeast.
We found that Zfp289 appears to have similar function, as it acti-
vates ARF-1-GTPase activity. The intracellular localization ex-
periments in SCp2 cells confirmed that Zfp289 functions pre-
dominantly in the cytoplasm, and in the majority of the cells,
Zfp289-GFP fusion proteins seemed to be concentrated around
the perinuclear region.

When we compared mRNA expression of Zfp289 with that of
Id-1 in the multiple tissue Northern blot, we found a direct
correlation in five out of seven tissues (Fig. 3). The liver
exhibited a relatively low level of Id-1 message compared with
that of Zfp289, while in lung, Id-1 expression was quite high as
compared with that of Zfp289. In these tissues, there may be
additional or other types of controls than Id-1 for regulating
Zfp289 gene expression. However, Zfp289 expression paralleled
that of Id-1 during mammary gland development, with
Zfp289 expressed during ductal (virgin) as well as lobulo-
alveolar (pregnant) morphogenesis, when there is extensive
proliferation of mammary epithelial cells. Its down-regulation
followed the decrease in Id-1 expression in differentiated,
growth-arrested lactating epithelial cells.

The data presented in Fig. 1, A and B, provide indirect
evidence that Zfp289 mRNA expression may be controlled by
Id-1 levels. To establish this relationship directly, it will be
necessary to sequence the Zfp289 promoter, to determine the
presence of E-box motifs and to isolate the Id-1-interacting
bHLH proteins, work now in progress. Nevertheless, this novel
zinc finger protein Zfp289 appears to mediate some of the
Id-1-dependent phenotypic effects on mouse mammary epithe-
rial cells. Functional analysis using retroviral-mediated trans-
faction in SCp2 cells indicates that it may be an important
mediator of Id-1-dependent S-phase entrance (Fig. 6B), a
conclusion consistent with the known function of the yeast
protein. The increased expression of Zfp289 may confer an advantage in
cell cycle entrance to Zfp289-transfected cells in comparison
with control cells. This may represent an example of the in-
volvement of ARF-GAP proteins in many fundamental cellular
processes such as cell growth and survival, as well as vesicular
trafficking and cytoskeletal organization.

Although we have no direct evidence that Zfp289 plays a role
in migration or invasion of cells, this question should remain
open. Besides the role of the yeast homologue Gcs-1 in the
cytoskeletal organization, it has been found that members of
the GTPase family (such as Rho and Rac) and their activators
can regulate cell migration through their control of actin po-
lymerization and cytoskeletal distribution (29). Our negative
results obtained from invasion assays in Zfp289-transfected
cells may be due to the fact that constitutive expression of
Zfp289 alone is not sufficient to induce the invasive phenotype,
an event that may also require the induction of some other
genres, such as matrix metalloproteinases.

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REFERENCES

1. Massari, M. E., and Murre, C. (2000) Mol. Cell. Biol. 20, 429–440
2. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L., and Weintraub, H.
(1990) Cell 61, 49–59
3. Cross, J. C., Flannery, M. L., Blanar, M. A., Steingrimsson, E., Jenkins, N. A.,
Copoland, N. G., Rutter, W. J., and Werb, Z. (1995) Development (Camb.)
121, 2513–2523
4. Lister, J., Forrestor, W. C., and Baron, M. H. (1995) J. Biol. Chem. 270,
17939–17946
5. Wilson, R. B., Kiledjian, M., Shen, C.-P., Benezra, R., Zwollo, P., Dymecki,
S. M., Desiderio, S. V., and Kadesch, T. (1991) Mol. Cell. Biol. 11, 6185–6191
6. Sun, X. H. (1994) Cell 79, 893–900
7. Kreidler, B. L., Benezra, R., Rovera, G., and Kadesch, T. (1992) Science
253, 1700–1702
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8. Desprez, P. Y., Hara, E., Campisi, J., and Bissell, M. J. (1995) Mol. Cell. Biol. 15, 3398–3404
9. Desprez, P. Y., Lin, C. Q., Thomasset, N., Symson, C. J., Bissell, M. J., and Campisi, J. (1998) Mol. Cell. Biol. 18, 4577–4588
10. Lin, C. Q., Singh, J., Murata, K., Itahana, Y., Pannell, S., Liang, S. H., Gillett, C. E., Campisi, J., and Desprez, P. Y. (2000) Cancer Res. 60, 1332–1340
11. Norton, J. D., Deed, R. W., Craggs, G., and Sablitzky, F. (1998) Trends Cell Biol. 8, 58–65
12. Hara, E., Uzman, J. A., Dimri, G. P., Nehlin, J. O., Testori, A., and Campisi, J. (1996) Dev. Genet. 18, 161–172
13. Nakajima, T., Yamada, M., Shiozaki, K., Murata, K., Suzuki, M., Tominaka, Y., and Oda, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10580–10585
14. Norton, J. D., and Atherton, G. T. (1998) Mol. Cell. Biol. 18, 2371–2381
15. Prabhu, S., Ignatova, A., Park, S. T., and Sun, X. H. (1997) Mol. Cell. Biol. 17, 5888–5896
16. Tanaka, K., Pracyk, J. B., Takeda, K., Yu, Z. X., Ferrans, V. J., Deshpande, S. S., Ozaki, M., Hwang, P. M., Lowenstein, C. J., Irani, K., and Finkel, T. (1998) J. Biol. Chem. 273, 25922–25928
17. Lyden, D., Young, A. Z., Zagrave, D., Yan, W., Gerald, W., O’Reilly, R., Baden, B. L., Hynes, R. O., Zhang, Y., Manova, K., and Benezra, R. (1999) Nature 401, 670–677
18. Desprez, P. Y., Roskelley, C., Campisi, J., and Bissell, M. J. (1993) Mol. Cell Differ. 1, 99–110
19. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
20. Maniatis, T., Frisch, E. F., and Sambrook, J. (1989) Molecular Cloning: A Laboratory Manual, Second Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Holzinger, A., Phillips, K. S., and Weaver, T. E. (1996) BioTechniques 20, 804–806
22. Goldberg, J. (1999) Cell 96, 893–902
23. Ireland, L. S., Johnston, G. C., Drobak, M. A., Dhillon, N., DeMaggio, A. J., Hoekstra, M. F., and Singer, R. A. (1994) EMBO J. 13, 3812–3821
24. Hraba-Beneve, S., and Kress, M. (1989) Nucleic Acids Res. 17, 2445–2461
25. Poon, P. P., Wang, X., Rotman, M., Huber, I., Cukierman, E., Cassel, D., Singer, R. A., and Johnston, G. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10071–10077
26. Blader, I. J., Cope, M., Jackson, T. R., Profit, A. A., Greenwood, A. F., Drubin, D. G., Prestwich, G. D., and Theilbert, A. B. (1999) Mol. Biol. Cell 10, 581–596
27. Wang, X., Hoekstra, M. F., DeMaggio, A. J., Dhillon, N., Vancura, A., Kuret, J., Johnston, G. C., and Singer, R. A. (1996) Mol. Cell. Biol. 16, 5375–5385
28. Antonny, B., Huber, I., Paris, S., Chabre, M., and Cassel, D. (1997) J. Biol. Chem. 272, 30848–30851
29. Hall, A. (1986) Science 279, 509–514