BRCA1/BARD1 Ubiquitinate Phosphorylated RNA Polymerase II*

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The breast- and ovarian-specific tumor suppressor BRCA1, when associated with BARD1, is an ubiquitin ligase. We have shown here that this heterodimer ubiquitinates a hyperphosphorylated form of Rpb1, the largest subunit of RNA polymerase II. Two major phosphorylation sites have been identified in the Rpb1 carboxyl terminal domain, serine 2 (Ser-2) or serine 5 (Ser-5) of the YSPTSPS heptapeptide repeat. Only the Ser-5 hyperphosphorylated form is ubiquitinated by BRCA1/BARD1. Overexpression of BRCA1 in cells stimulated the DNA damage-induced ubiquitination of Rpb1. Similar to the in vitro reaction, the stimulus of Rpb1 ubiquitination by BRCA1 in cells occurred only on those molecules hyperphosphorylated on Ser-5 of the heptapeptide repeat. In vitro, the carboxyl terminus of BRCA1 (amino acids 501–1863) was dispensable for the ubiquitination of hyperphosphorylated Rpb1. In cells, however, efficient Rpb1 ubiquitination required the carboxyl terminus of BRCA1, suggesting that interactions mediated by this region were essential in the complex milieu of the nucleus. These results link the BRCA1-dependent ubiquitination of the polymerase with DNA damage.

BRCA1, the breast- and ovarian-specific tumor suppressor protein, has been found to regulate a number of processes central to the normal function of the cell, including transcription, chromatin dynamics, homologous recombination, and other forms of DNA damage repair (1, 2). Because BRCA1 has been found associated with a wide range of proteins involved in these processes, it may function as a scaffold, organizing effector proteins in a context-dependent manner. However, when BRCA1 is associated with the BARD1 protein, it is also an enzyme, an E3 ubiquitin ligase (3, 4). The realization that BRCA1 is an enzyme establishes the necessity of identifying its substrates in order to understand how the ubiquitination activity impacts these processes in the cell.

BRCA1 and BARD1 are associated with the messenger RNA-synthesizing polymerase in a complex known as the RNA polymerase II holoenzyme (holo-pol)1 (5–7). One function for BRCA1 in this holo-pol complex appears to be as a coactivator of transcription, because it has been shown that BRCA1 stimulates the activation signal of p53, NF-κB, and others (8–13). Previously, we modeled that the BRCA1 and BARD1 in the holo-pol complex may ubiquitinate the transcribing RNA polymerase II (RNAPII) when it encounters DNA damage, and we also suggested that this ubiquitination event would stimulate the repair process (14, 15).

Rpb1 is the largest subunit of RNAPII, and its carboxyl-terminal domain (CTD) is highly conserved, consisting of multiple repeats (27 in budding yeast, 52 in humans) of the heptapeptide YSPTSPS. Serines 2 (Ser-2) and 5 (Ser-5) of multiple repeats are phosphorylated co-transcriptionally, Ser5*p predominating at the promoter and Ser2*p in the coding sequence (16, 17). In response to DNA damage Rpb1 is also ubiquitinated, an event associated with changes in concentration of both the hypophosphorylated and the hyperphosphorylated Rpb1 (18). In budding yeast, the Rsp5 E3 ligase ubiquitates Rpb1 independent of its phosphorylation state (19, 20). In higher eukaryotes the ubiquitin ligase(s) that mediate this modification of RNAPII are unknown, and it is possible that multiple factors mediate the reaction. Because BRCA1 and BARD1 are associated with RNAPII in the holo-pol complex (6), BRCA1 is a reasonable candidate for the RNAPII ubiquitin ligase. In addition, after DNA damage BRCA1 and BARD1 also associate with the polyadenylation cleavage factor CstF (21), known to interact with RNAPII via Rpb1 hyperphosphorylated on Ser-2 (Ser2*p) of the YSPTSPS heptapeptide repeats (22, 23). These results led us to speculate that a substrate for BRCA1-dependent ubiquitination could be the Ser2*p form of Rpb1.

In these experiments we tested whether BRCA1 in association with BARD1 could ubiquitinate RNAPII. We found that hyperphosphorylated RNAPII serves as a substrate for the BRCA1-dependent ubiquitination activity, and we found that overexpression of BRCA1 in cells stimulates the DNA damage-induced ubiquitination of hyperphosphorylated RNAPII. Strikingly, the ubiquitination reaction, when tested both in vitro and in vivo, was enhanced not by Ser2*p of the heptapeptide

1 The abbreviations used are: holo-pol, RNA polymerase II holoenzyme; BRCA1, breast cancer gene 1; BARD1, BRCA1-associated RING domain protein 1; CTD, Rpb1 carboxyl-terminal domain; GST, glutathione S-transferase; RNAPII, RNA polymerase II; Rpb1, RNA polymerase II subunit 1; Ser2*p, phosphorylated serine 2 of YSPTSPS; Ser5*p, phosphorylated serine 5 of YSPTSPS; HEK, human embryonic kidney; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein; E3, ubiquitin-protein isopeptide ligase; HA, hemagglutinin.

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repeat but rather by Ser5* p. These results thus identify a substrate for ubiquitination by BRCA1/BARD1 that is correlated with the cellular response to DNA damage.

MATERIALS AND METHODS

**Protein Purification**—The expression and purification of BRCA1 and BARD1 from baculovirus-infected insect cells has been described, along with a description of the purification of the ubiquitination factors E1 and UbcH5c (22). The core RNAPII was purified from calf thymus using an established protocol (25). The budding yeast Rpb1 CTD was expressed as a hexahistidine and GST fusion (26) and purified by nickel-nitriilotriacetic acid chromatography using standard techniques. Ubiquitin was obtained from a commercial vendor (Sigma).

The yeast Kin28, Ctk1, and Srb10 kinases were each expressed in Saccharomyces cerevisiae as HA-tagged fusion proteins. Active kinases were purified by immunoprecipitation using the 12CA5 monoclonal antibody specific for the HA tag (27, 28).

Human TFIIH was purified from HEK-293 cells as described (29). In brief, ~10^12 cells were collected over a period of several months, and a whole cell extract was prepared for each. The whole cell extracts were bound to a BioXrest70 matrix at 0.15 M KOAc in buffer A (20 mM Hepes, pH 7.9, 1 mM EDTA, 5% glycerol, 3 mM dithiothreitol), washed at 0.3 M KOAc, and the peak was collected at 1.5 M KOAc. At each column step, TFIIH-containing fractions were identified by Western blotting using antibodies specific to the 89-kDa ERCC-3 subunit of TFIIH. The 1.5 M KOAc peak fraction was dialyzed to 0.1 M KCl in buffer A, bound to a DEAE fast flow matrix, and the protein peak at 0.3 M KCl was collected and dialyzed to 0.1 M KCl. The protein was bound to a 2-ml BioScale-Q column (Bio-Rad Laboratories), and protein was eluted with a 0.1- to 1.0-M KCl TFIIH-containing fractions were subjected to gel filtration using a Superdex-200 (HR16/60; Amersham Biosciences) column in 0.3 M KCl in buffer A. The TFIIH migrated at a volume consistent with a 700-kDa complex, and samples were dialyzed in 0.1 M KCl in buffer A.

**In Vivo Ubiquitination Assay**—Purified RNAPII (10 ng) or 300 ng of GST/CTD reaction were phosphorylated using purified human TFIIH or 12CA5, Ctk1, or HA-Rub2 antibodies (30). After TFIIH complexes using the following reaction conditions: 10 mM HEPES (pH 7.9), 0.5 mM EDTA, 5% glycerol, 60 mM KCl, 5 mM MgCl2, 5 mM NaF, 10 µCi of [γ-32P]ATP. 32P-labeled RNAPII was then added to ubiquitination reactions that contained 100 ng of FLG-BRCA1/BARD1 (25 ng) or truncations of BRCA1 co-purified with BARD1 (24), 100 ng of His* E1 ubiquitin ligase (40 ng), 1.5 µg of His* UbcH5c (4 µg), and 2 µg of ubiquitin (12 µM) in the following reaction conditions: 10 mM HEPES, pH 7.9, 5% glycerol, 60 mM KCl, 5 mM MgCl2, 5 mM NaF, 2 mM ATP. All reactions were incubated at 37 °C for 30 min. The reactions were stopped by addition of sample buffer and resolved by SDS-PAGE.

**Plasmid Construction**—pcDNA3-HA-BRCA1(Δ775–1292)-C61G was constructed as follows. The plasmid pcDNA3-HA-BRCA1(Δ775–1292) has been described previously (30). A mutation C61G was amplified from an adenovirus shuttle vector that expresses full-length HA-BRCA1-C61G (31). PCR from this template used the primers 5'-ATACATCCGAAGCCGCGAGCAGTC-3' and the HindIII site and 5'-TCGGATATGCTGCTTGCTTTG-3' that is located in 3'-side of the EcoRI site of BRCA1. The PCR product was subcloned into the HindIII and EcoRI sites of pcDNA3-HA-BRCA1(Δ775–1292). pcDNA3-HA-BRCA1(Δ775–1292) was constructed as follows. A fragment was amplified from the template pcDNA3-HA-BRCA1 using the mutagenic primers 5'-GCCCCTTCAACACAAGGCCGACAGTC-3' and a downstream, vector-encoded primer 5'-TGACACTATAGAAT-3' to amplify a fragment encoding BRCA1 amino acids 1293–1863 containing the M1775R substitution. The second PCR product was subcloned into the NheI and EcoRV sites of pcDNA3-HA-BRCA1(Δ775–1292), then replacing the wild-type sequence.

pcDNA3-HA-BRCA1(Δ775–1292), [Δ1527–1863]) was constructed as follows. The fragment containing HA-BRCA1 sequences up to residue 1526 was generated by digestion of pcDNA3-HA-BRCA1(Δ775–1292) with HindIII and SacI and then inserted into the HindIII and EcoRV sites of the vector backbone for pcDNA3-HA-BRCA1(Δ775–1292).

pcMV-Myctagged ubiquitin was constructed as follows. Ubiquitin was amplified from cDNA of HaLa cells as a template using the primers 5'-GCCGAGATCTCGAGATCTTATGTGACAGAAC-3' and 5'-GCCGAGCTAGCTACACCTTCTACCAAGCAGGC-3' that contain 5'-EcoRI site and 3'-XhoI site. The PCR product was then subcloned into the pcMV-Myct vector (CloneTech). All constructs were verified by DNA sequence.
purified E1, E2 UbcH5c, E3 BRCA1/BARD1, and ubiquitin (lane 3). E1 (lane 2), E2 UbcH5c (lane 4), E3 BRCA1/BARD1 (lane 5), and ubiquitin (lane 6). C, reactions as in panel B were repeated except that TFIH-phosphorylated GST-CTD was used in place of RNAPII.

The specificity of the BRCA1/BARD1 E3 ligase in this reaction was striking. If the heterodimer was simply binding to and ubiquitinating a long polypeptide with multiple negative charges, as in the hyperphosphorylated CTD, then we would expect little or no preference for either the Ser2*p or Ser5*p forms. Instead, the ubiquitination by BRCA1/BARD1 was specific for the Ser5*p CTD. In binding experiments using the purified BRCA1/BARD1 and purified RNAPII, we found that the BRCA1 bound to RNAPII independent of phosphorylation (Fig. 2B, right panel). This result was not surprising because it is known that BRCA1 binds to Rpb2 and Rpb12 of RNAPII (32). However, when comparing the effectiveness of the purification of RNAPII on a BRCA1/BARD1 affinity matrix, the recovery of the Ser5*p-RNAPII was more complete than was observed for the hypophosphorylated form (Fig. 2B, left panel). Thus, binding alone did not specify the ubiquitination substrate, but Ser5-specific phosphorylation enhanced both the level of binding and of ubiquitination by BRCA1/BARD1. Note that the Ser5*p form of the CTD is observed at the promoter, whereas the Ser2*p form of the CTD is observed at the 30 end of the transcript when the polymerase is at the elongation phase (29). If the Ser2*p form of the CTD is observed at the 30 end of the transcript, then we would have expected no preference for either the Ser2*p or Ser5*p forms. Instead, the ubiquitination by BRCA1/BARD1 was specific for the Ser5*p CTD. In binding experiments using the purified BRCA1/BARD1 and purified RNAPII, we found that the BRCA1 bound to RNAPII independent of phosphorylation (Fig. 2B, right panel). This result was not surprising because it is known that BRCA1 binds to Rpb2 and Rpb12 of RNAPII (32). However, when comparing the effectiveness of the purification of RNAPII on a BRCA1/BARD1 affinity matrix, the recovery of the Ser5*p-RNAPII was more complete than was observed for the hypophosphorylated form (Fig. 2B, left panel). Thus, binding alone did not specify the ubiquitination substrate, but Ser5-specific phosphorylation enhanced both the level of binding and of ubiquitination by BRCA1/BARD1. Note that the Ser5*p form of the CTD is observed at the promoter, whereas the Ser2*p form of the CTD is associated with transcription elongation (17). Thus, the Ser5*p-specific modification of RNAPII by BRCA1/BARD1 is not consistent with targeting the elongating polymerase for ubiquitination.

BRCA1 Truncated from the Carboxyl Terminus Ubiquiti-

nated Phosphorylated RNAPII in Vitro—The carboxyl terminus of BRCA1 (amino acids 1650–1863) associates with RNAPII via interactions with Rpb2, Rpb12, and rho-Rpb1 subunits (7, 32). To determine whether the carboxyl terminus is required to mediate ubiquitination of RNAPII in vitro, we purified carboxyl-terminal truncations of BRCA1 in heterogeneous complex with full-length BARD1 (24). In addition to full-length FLAG-tagged BRCA1 (1–1863), FLAG-tagged BRCA1 (1–1852), BRCA1 (1–1527), BRCA1 (1–1000), and BRCA1 (1–500) were coexpressed with untagged BARD1 and purified. A DN-BRCA1 construct (301–1863) lacking the amino-
domain and was thus expected to lack ubiquitination activity. The terminus of BRCA1 and BARD1 reveal extensive interaction action with BARD1 (3, 4), and structural studies of the amino nition activity of BRCA1 is significantly potentiated by its inter- ubiquitination activity of BRCA1, and the isolated RING do- required for ubiquitination of RNAPII (Fig. 3B). The absence of ubiquitination was evident when BRCA1 lacking BARD1 was included in reactions (Fig. 3C). Under these more sensitive conditions, weak ubiquitination was observed for all constructs tested with the exception of...
HA-BRCA1(ΔM)-expressing cells a significant increase in the intensity of the slowly migrating band was observed (lane 7) that we interpret to be multiply ubiquitinated RNAPII. These results indicate that overexpression of BRCA1(ΔM) stimulated ubiquitination of Ser5*p-RNAPII. The HA monoclonal antibody that specifically binds to Ser5*p RNAPII was not detected (data not shown). These results were consistent with the in vitro experiments (Fig. 2) in which Ser-5 phosphorylation of the RNAPII CTD specifically stimulated its ubiquitination by BRCA1/BARD1. These results were also consistent with the previously established ubiquitination of Ser-5-phosphorylated RNAPII after UV-induced DNA damage (18, 40).

The consequences of BRCA1-dependent ubiquitination are unclear. BRCA1/BARD1 have been shown to direct the linkage of ubiquitin chains via either lysine 6, lysine 48, or lysine 63 isopeptide bonds (4, 42). Appending ubiquitin chains via lysine isopeptide bonds (4, 42).
48 target the substrate for proteasome-mediated degradation; thus BRCA1/BARD1 ubiquitination may in some cases lead to protein degradation. We tested whether inhibition of the proteasome, using MG132, could stabilize the ubiquitinated phospho-RNAPII. Proteasome inhibition resulted in longer chains of ubiquitin appended on the Rpb1 subunit of RNAPII (Fig. 4C, lane 4, top panel), suggesting that BRCA1-dependent ubiquitination may cause degradation of RNAPII. Interestingly, UV irradiation of cells resulted in a shift in the polyubiquitin pattern from RNAPII to RNAPII (Fig. 4C, bottom panel), a phenomenon that has been observed previously (18). Although quantitation using two different antibodies in immunoblots is imprecise, this result suggests that phosphorylation of Rpb1 to Ser5* is a generalized response after DNA damage. Although proteasome inhibition stabilized the recovery of ubiquitinated RNAPII (lanes 3 and 4), the amount of RNAPII in the lysate was not markedly increased (Fig. 4C, lanes 3–4, bottom panel). We infer from this result that only a fraction of the total RNAPII is targeted for degradation following BRCA1-dependent ubiquitination.

Repeating the experiment, but using the H14 antibody to immunoprecipitate the RNAPII and the anti-Myc antibody on immunoblots to detect the ubiquitin, revealed that HA-BRCA1(ΔM) expression stimulated the appearance of ubiquitinated RNAPII (Fig. 4D, lane 2). As in panel B, expression of HA-BRCA1(ΔM) in UV-irradiated cells resulted in the recovery of higher levels of ubiquitinated RNAPII (Fig. 4D, lane 4). Compared with anti-Myc ubiquitin immunoprecipitation, use of the H14 antibody reproducibly yielded lower amounts of ubiquitinated RNAPII, even after UV irradiation. We interpret this lower level to be due to less effective immunoprecipitation reactions with the latter antibody.

We have previously shown that BRCA1 is a component of RNAPII holo-pol, and the carboxyl terminus of BRCA1 is important for this association (5, 6). In the in vitro assays in this study (Fig. 3), the carboxyl terminus of BRCA1 was not required for ubiquitination of the polymerase. However, in the complicated environment of a cell, the carboxyl-terminal-mutated BRCA1 might not associate with the polymerase and thus not ubiquitinate it. We examined whether the carboxyl terminus of BRCA1 affected ubiquitination of phospho-RNAPII in tissue culture cells. We found that overexpression of BRCA1 lacking its carboxyl terminus resulted in only background levels of ubiquitinated RNAPII (Fig. 5B, compare lanes 1–4). We thus conclude that in the cells the carboxyl terminus of BRCA1 is important for the UV damage-induced ubiquitination of RNAPII.

We also tested whether a specific missense mutation associated with breast cancer affects the ubiquitination of RNAPII. The disease-associated missense mutation M1775R in the BRCT domain of the carboxyl terminus of BRCA1 ablates the double strand break repair and transcription activation function of BRCA1 (43). BRCA1 proteins containing the M1775R mutation do not bind to histone deacetylases (44), BACH1 (45), and the transcriptional co-repressor CtBP (46, 47). As shown in Fig. 5B, expression of BRCA1 with M1775R did not stimulate the ubiquitination of phosphorylated RNAPII (Fig. 5B, lane 5, top panel). Although the mutation of BRCA1 at residue M1775R decreases the stability of the protein (48), the expression level of the HA-BRCA1(ΔM-M1775R) was equal to that of HA-BRCA1(ΔM) (Fig. 5B, middle panel). Furthermore, the M1775R mutation disrupted BRCA1 binding to RNAPII (Fig. 6). In transfected cells, immunopurification of HA-BRCA1(ΔM) also purified Ser5*p Rpb1 (Fig. 6, lane 2). Deletion of the carboxyl terminus of BRCA1 or the BRCA1 protein containing a missense mutation resulted in significantly decreased binding to RNAPII (Fig. 6, lanes 3 and 4). Thus, an intact carboxyl terminus was required for BRCA1 to bind to RNAPII. These data suggest that ubiquitination of phosphorylated RNAPII by BRCA1 in response to DNA damage requires an intact BRCT domain.

The active site of BRCA1 for ubiquitin ligase activity is encoded in the RING domain of the amino terminus of the protein. Missense mutation of one of the zinc-coordinating residues, C61G, results in an inactive E3 ubiquitin ligase even in the presence of wild-type BARD1 (3, 34, 35). In patients, inheritance of this missense mutation is associated with breast cancer (49, 50). Expression of HA-BRCA1(ΔM) containing the C61G missense mutation did not stimulate the ubiquitination of phosphorylated RNAPII (Fig. 5C, top panel).

The experiment in Fig. 5C was repeated, but the immunoprecipitating antibody was the Ser5*p-specific H14, and ubiquitinated species were detected using the Myc-specific antibody on immunoblots. As before, we observed that HA-BRCA1(ΔM) expression stimulated the recovery of ubiquitinated RNAPII (Fig. 5D, lane 3). Further, expression of BRCA1 variants containing the missense mutation C61G (lane 4) or a carboxyl-terminal truncation (lane 5) failed to stimulate the ubiquitination of RNAPII. As in Fig. 4D, this immunoprecipitation reaction was weaker than when the Myc antibody was used, and we only detected the ubiquitinated species when HA-BRCA1(ΔM) was expressed. Taken together, the data in Figs. 4 and 5 indicate that BRCA1 stimulates the ubiquitination of Ser5*p RNAPII after UV irradiation.

**DISCUSSION**

Identification of the substrates for BRCA1-dependent ubiquitination activity is important for understanding how mutation of BRCA1 is associated with loss of tumor suppression activity. The currently identified substrates include histone proteins, p53, Fanconi anemia protein D2, and centrosomal proteins including NPM1 and γ-tubulin (24, 51–54). Among these, only the modification of γ-tubulin by BRCA1/BARD1 has been shown to affect the biology of breast cells. It has been shown that failure to ubiquitinate γ-tubulin results in centrosome amplification (24). The BRCA1/BARD1 proteins are known to regulate multiple processes in the cell, including transcription, DNA repair, and centrosome dynamics (5, 55–59). Although the ubiquitination of γ-tubulin may in part ex-
plain the BRCA1-dependent regulation of centrosome dynamics, it was unclear whether the BRCA1-dependent ubiquitination activity also regulates the transcription and DNA repair function of BRCA1.

We had proposed that the BRCA1-dependent ubiquitination activity may function in DNA repair by modification of RNAPII that transcribes DNA near a lesion (14, 15). This proposed role for BRCA1 in transcription-coupled repair could be important following UV damage or double strand breaks. One prediction of this model was that BRCA1/BARD1 ubiquitination activity would be targeted to the elongating, hyperphosphorylated form of RNAPII. Actively transcribing RNAPII is phosphorylated on Ser-5 proximal to the promoter and on Ser-2 further downstream (23). Thus, the principal form of RNAPII that elongates through a gene is the Ser2*p form, which we now show is not a substrate for BRCA1/BARD1. The model that BRCA1-dependent ubiquitination directly links transcription elongation to repair is thus not supported. Instead, we found that Ser-5 phosphorylation of RNAPII is a generalized response to UV irradiation, and BRCA1-dependent ubiquitination modifies the RNAPII. It has been observed that transcriptionally engaged RNAPII does become phosphorylated on Ser-5 by the action of extracellular signal-regulated kinases 1 and 2 (60). The data are most consistent with a model whereby DNA damage causes phosphorylation of a subpopulation of RNAPII, followed by ubiquitination by BRCA1/BARD1 and subsequent degradation at the proteasome.

In these experiments we found that overexpression of BRCA1 in cells could stimulate the damage-induced ubiquitination of RNAPII. When we inhibited BRCA1 expression by transfection of short interfering RNA specific for BRCA1, we did not observe a decrease in ubiquitination of RNAPII. When we inhibited BRCA1 expression by BRCA1 in cells could stimulate the damage-induced ubiquitination of RNAPII. When we inhibited BRCA1 expression by transfection of short interfering RNA specific for BRCA1, we did not observe a decrease in ubiquitination of RNAPII. When we inhibited BRCA1 expression by transfection of short interfering RNA specific for BRCA1, we did not observe a decrease in ubiquitination of RNAPII. When we inhibited BRCA1 expression by transfection of short interfering RNA specific for BRCA1, we did not observe a decrease in ubiquitination of RNAPII.
and Livingston, D. M. (1997) Cell 88, 265–275
56. Snouwaert, J. N., Gowen, L. C., Latour, A. M., Mohn, A. R., Xiao, A., DiBiase, L., and Koller, B. H. (1999) Oncogene 18, 7900–7907
57. Moynahan, M. E., Chiu, J. W., Koller, B. H., and Jasin, M. (1999) Mol. Cell 4, 511–518
58. Hsu, L. C., and White, R. L. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 12983–12988
59. Xu, X., Weaver, Z., Linke, S. P., Li, C., Gotay, J., Wang, X. W., Harris, C. C., Ried, T., and Deng, C. X. (1999) Mol. Cell 3, 389–395
60. Inukai, N., Yamaguchi, Y., Kurooka, I., Yamada, T., Kamijo, S., Kato, J., Tanaka, K., and Handa, H. (2004) J. Biol. Chem. 279, 8190–8195
61. Svejstrup, J. Q. (2003) J. Cell Sci. 116, 447–451
