SUMO-1 Conjugation in Vivo Requires Both a Consensus Modification Motif and Nuclear Targeting*

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SUMO-1 is a small ubiquitin-related modifier that is covalently linked to many cellular protein targets. Proteins modified by SUMO-1 and the SUMO-1-activating and -conjugating enzymes are located predominantly in the nucleus. Here we define a transferable sequence containing the WKKXE motif, where W represents a large hydrophobic amino acid, that confers the ability to be SUMO-1-modified on proteins to which it is linked. Whereas addition of short sequences from p53 and IkBα, containing the WKKXE motif, to a carrier protein is sufficient for modification in vitro, modification in vivo requires the additional presence of a nuclear localization signal. Thus, protein substrates must be targeted to the nucleus to undergo SUMO-1 conjugation.

SUMO-1 is a small ubiquitin-related modifier (also known as sentrin, GMP1, UBL1, PIC1, or SMT3 in yeast) that has been found covalently conjugated to various cellular proteins (for reviews see Refs. 1–3). Several substrates for SUMO-1 have been reported: the RanGTPase-activating protein (RanGAP1) (4, 5) and Ran-binding protein 2 (6) implicated in nucleocytoplasmic trafficking; the promyelocytic leukemia protein (PML) and Sp100 (7) found in subnuclear structures known as PML oncopgenic domains or PODs; the IkBα inhibitor of the transcription factor nuclear factor κB, implicated in the control of immune and inflammatory responses (8); and the tumor suppressor protein p53 (9, 10). Consequently, “SUMOylation” plays a role in multiple vital cellular processes such as oncogenesis, cell cycle control, apoptosis, and response to virus infection.

SUMO-1 is conjugated to a target protein by a pathway that is distinct from but analogous to ubiquitin conjugation. Like ubiquitin, SUMO-1 is proteolytically processed to expose its mature C terminus by recently described SUMO-1-specific proteases variously called Ulp1 and Ulp2 in yeast (11, 12) or SENP1 and SUSP-1 in vertebrates (13–15). Ulp1, Ulp2, and SENP1, but not SUSP-1, are capable of both deconjugating SUMO-1 from modified proteins and removing four amino acids from the C terminus of the 101-amino acid SUMO-1 precursor to generate the mature 97-amino acid form. SUMO-1 addition is accomplished by a thioester cascade, with SUMO-1 first being activated by a heterodimeric SUMO-1-activating enzyme (SAE) that adenylates the C-terminal glycine of SUMO-1 (16–19) before catalyzing the formation of a thioester bond between the C terminus of SUMO-1 and a cysteine residue in SAE. In a transesterification reaction SUMO-1 is transferred from the SAE to the SUMO-1-conjugating enzyme Ubc9, which catalyzes the formation of an isopeptide bond between the C terminus of SUMO-1 and the e-amino group of a lysine residue of the target protein (6, 20–23). Ubc9 is required for cell cycle progression in yeast (24). Unlike ubiquitin conjugation, SUMO-1 modification of target proteins in vitro is not dependent on the equivalent of an E3 protein ligase (17, 19).

Here, we demonstrate that a short sequence containing the consensus WKKXE, where W represents a large hydrophobic amino acid, constitutes a transferable signal that confers the ability to be modified with SUMO-1 on proteins to which it is linked. The predominantly nuclear localization of both subunits of the SAE, Ubc9 and SUMO-1, suggest that SUMOylation is a nuclear process. We demonstrate that heterologous proteins carrying the SUMO-1 consensus modification sequences present in IkBα and p53 are only conjugated to SUMO-1 in vitro when a nuclear localization signal (NLS) is also present. These data suggest that protein substrates must be targeted to the nucleus to undergo SUMO-1 conjugation and allow us to propose that this modification may be involved in regulating multiple processes in the nucleus.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cells were transfected by electroporation as described previously (25). For immunofluorescence analysis 2 μg of plasmid were transfected in 1 × 106 HeLa cells. For nickel bead purification, 10 μg of each plasmid DNA encoding pyruvate kinase (PK) fusions and His6-SUMO-1 were transfected in 1 × 106 HeLa cells. To increase efficiency of protein expression, no DNA carrier was used in cotransfections. After transfection, cells were seeded in 75 cm2 flasks. One-twentieth of transfected cells were seeded in a separated plate (to control protein input), and incubation was continued for 24 h.

Plasmids and DNA Manipulations—Plasmids encoding His6-SUMO-1, HA-SUMO-1, SV5-SAE1, HA-SAE2, and SV5-Ubc9 were reported previously (8, 9, 17). pcDNA3 plasmids encoding Myc-tagged PK and NLS-PK were described previously (26). cDNA encoding the 1–26 fragment of IkBα was obtained by polymerase chain reaction using as template IkBα wild type and IκBα21R,22R-encoding plasmids (27) to generate PK-IκBα(1–26) and PK-IκBα(1–26)-KR. cDNAs encoding 361–393 and 361–393 KR of p53 were subcloned from previously described constructs (9). Polymerase chain reaction fragments and synthetic oligonucleotides encoding the 381–391 fragment of p53, the 16–26 fragment of IκBα, the 519–529 fragment of human RanGAP1, the 99–109 fragment of adenovirus type 2 E1B, and the 485–495 fragment of PML, as well as Lys to Arg versions, were cloned in BamHI and XbaI

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restriction sites of the PK vector. The same cDNAs were subcloned into BamHI and XhoI restriction sites of the NLS-PK vector that contains a polylinker, KpnI-BamHI-EcoRV-XhoI, to generate NLS-PK versions.

**Immunofluorescence Microscopy**—Indirect immunofluorescence analysis was performed as described previously (28). Monoclonal antibodies anti-HA (Babco), anti-SV5 (Dr. R. Randall, University of St. Andrews), and anti-Myc (9E10) were applied for 30 min followed by a 30-min incubation with fluorescein isothiocyanate-conjugated donkey anti-mouse IgG (Jackson). Coverslips were mounted in Mowiol (Hoechst, Frankfurt, Germany). Images were acquired on a DMIRE fluorescence microscope (Leica) with a CCD camera (Princeton).

**In Vitro SUMO-1 Assay**—In vitro transcription/translation (Promega) and SUMO-1 conjugation assays were performed as reported (8).

**Preparation of Cell Extracts and Immunoblotting**—Cells were harvested in 100 μl of lysis buffer for Western blot analysis (8). His6-SUMO-1 conjugates were purified as described (9). Proteins were resolved by electrophoresis in 8.5% polyacrylamide gels containing SDS, transferred to polyvinylidene difluoride membranes (Sigma) by electroblotting, and processed for Western blotting as reported previously (9). Primary monoclonal antibody anti-Myc (9E10) was obtained from Dr. R. E. Randall. Horseradish peroxidase-conjugated anti-mouse IgG was purchased from Amersham Pharmacia Biotech. An enhanced chemiluminescence detection system was used to detect specific antigen-antibody interactions (Amersham Pharmacia Biotech).

### RESULTS

**Nuclear Distribution of the SUMO-1 Conjugation Pathway Enzymes**—It has been previously reported that Ubc9 promotes the nuclear localization of a Ub-β-galactosidase fusion protein in Saccharomyces cerevisiae (24). Moreover, Ubc9 has been localized predominantly in the nucleus of T cells (29). To analyze the subcellular distribution of all the components of the SUMO-1 conjugation pathway, we transiently transfected HeLa cells with HA-tagged versions of SUMO-1 and SAE-2 and SV5-tagged versions of Ubc9 and SAE-1 (Fig. 1). As expected, SUMO-1 displayed nuclear distribution and accumulated in nuclear dot-like structures (30) (Fig. 1a). Using paraformaldehyde fixation, Ubc9 was localized mainly in the nucleus but also in the cytoplasm of transfected cells (Fig. 1b). However, when cells were fixed with 1:1 methanol/aceton, Ubc9 immunoreactive material was concentrated at the nuclear envelope (data not shown and Ref. 23). The SAE-1 and SAE-2 (Fig. 1c and d, respectively) subunits of the SAE presented an exclusively nuclear distribution that was not changed when both subunits were coexpressed or when cells were fixed by different methods (data not shown). Thus, the predominant nuclear localization of enzymes implicated in the SUMO-1 modification pathway suggests that this ubiquitin-like modification may take place in the nucleus.

**Sequence Requirement for SUMO-1 Modification**—To test the proposition that SUMO-1 modification takes place in the nucleus, we designed an experiment strategy in which a minimal SUMO-1 modification site, fused to a heterologous protein, is located in either the nucleus or the cytoplasm by virtue of the presence or absence of an NLS. Constructs were designed such that these could be tested for SUMO-1 conjugation in vitro, where there is no influence of compartmentalization, or expressed in vivo either in the nucleus or the cytoplasm. Analysis of the sequence of SUMO-1 conjugation sites in multiple proteins indicates that a short motif YXXE represents the primary site of SUMO-1 modification (8, 9, 31–33). To further define the sequence required for conjugation with SUMO-1, we designed a series of constructs containing 1×Bo N-terminal and p53 C-terminal modification sites fused to the C terminus of either a Myc-tagged version of PK or an equivalent construct containing the SV40 NLS (NLS-PK) (Fig. 2). [35S]Met-labeled PK and NLS-PK fusions generated by in vitro transcription and translation were assayed for SUMO-1 conjugation in vitro using the previously described assay (8). PK and NLS-PK fused to amino acids 1–26 and 16–26 of 1×Bo were conjugated with SUMO-1, whereas PK or NLS-PK alone were not (Fig. 2A and B). When lysine residues 21 of 1×Bo and 386 of p53 were changed to arginine (KR constructs), SUMO-1-modified forms of PK-1×Bo-KR and PK-p53-KR conjugates were not detected, indicating that SUMO-1 was conjugated specifically to the previously described lysine residues (8–10). To determine whether the 11-amino acid sequence required for conjugation with SUMO-1 could be further reduced, a series of synthetic oligonucleotides that specifies the...
human RanGAP1 (519–529) (11 amino acids), 520–528 (9 amino acids), 521–527 (7 amino acids), and 522–526 (5 amino acids) amino acid sequences was fused to the C terminus of PK to generate the corresponding PK-RanGAP1 constructs (Figs. 2C and 4A). Efficient SUMO-1 conjugation was observed with PK fusion encoding 11, 9, and 7 amino acids, whereas the efficiency of conjugation was reduced but still detectable with only 5 amino acids (Fig. 2C). Thus, SUMO-1 modification requires a core recognition motif of five amino acids, although flanking residues influence the efficiency of conjugation.

**SUMO-1 Conjugation in Vivo Requires Nuclear Targeting**—In most cells, SUMO-1 is found in conjugates with target proteins, and as such the pool of free SUMO-1 is limiting (8). At present, most cellular substrates reported to be conjugated with SUMO-1 show a nuclear distribution (PML, Sp100, and p53), shuttle between the nucleus and the cytoplasm (IκBα) (34, 35), or are associated to the nuclear pore complex (Ran-GAP1 and Ran-binding protein 2) (4–6). Cell fractionation analysis indicates that 80–90% of endogenous SUMO-1-conjugated proteins have a nuclear distribution (7, 36, and data not shown). To determine whether SUMO-1 conjugation requires nuclear targeting, the PK-IκBα and PK-p53 constructs were compared with NLS-PK-IκBα and NLS-PK-p53 constructs for SUMO-1 conjugation in vivo. The ability of the NLS-PK constructs to be conjugated with SUMO-1 in vitro is identical to the PK fusion counterpart (Fig. 2, A and B). As expected, all PK constructs were localized in the cytoplasm, and all NLS-PK constructs were localized in the nucleus of transfected cells (Fig. 3A). To detect forms of PK and NLS-PK modified by SUMO-1 in vivo, constructs specifying these proteins were cotransfected into HeLa cells with an expression plasmid for His6-SUMO-1 (9). His6-SUMO-1-conjugated proteins were isolated on nickel beads, and eluted proteins were analyzed by Western blotting with a monoclonal antibody recognizing the Myc tag. NLS-PK-IκBα-(1–26) and -(16–26) as well as NLS-PK-p53-(361–393) and -(381–391) are efficiently conjugated with SUMO-1 (Fig. 3, B and C, top). Under the same conditions, KR mutants were not modified. In contrast to NLS-PK constructs, PK counterparts were not modified. These results clearly indicate that nuclear localization is required for conjugation of proteins with SUMO-1.

**Intrinsic Substrate Activity of SUMO-1 Modification Motifs**—Although a short motif can direct SUMO-1 modification when transferred to a heterologous protein, the efficiency of SUMO-1 modification will be a combination of the intrinsic substrate activity of the motif and the environment of the motif in the native protein. To investigate the intrinsic substrate activity of a range of SUMO-1 modification motifs, we generated additional PK constructs (Fig. 4A) encoding amino acids 485–495 of PML (31, 37) and amino acids 99–109 of the human adenoviral protein E1B,2 which contain the lysine residue that is the site of SUMO-1 conjugation (Fig. 4A). 35S-labeled PK-RanGAP1-(519–529), PK-p53-(381–391), PK-IκBα-(16–26), PK-AdE1B-(49–109), and PK-PML-(485–495) were used as substrates for SUMO-1 conjugation in vitro (Fig. 4B). A range of substrate activities is evident (Fig. 4B), with PK-AdE1B and PK-PML being modified efficiently, PK-IκBα and PK-p53 being modified less efficiently, and PK-RanGAP1 being modified poorly. These differences were also observed in vivo when comparing NLS-PK fusions encoding PML, IκBα, and p53 sequences (Fig. 4C) in conditions where PK counterparts were poorly modified or not modified at all (data not shown). The differences observed in the conjugation of SUMO-1 to this range of substrates indicates that the precise sequence of the

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2 A. Errico and R. T. Hay, unpublished data.
The altered substrate is modified very poorly when compared to PK-PML construct, and the substrate activity was determined. Glu to Asp change was therefore made within the context of the residue was absolutely required for activity. The conservative sequence position were without consequence. Although most described in substrate activity, all of the other modifications at this position were tested as substrates for SUMO-1 conjugation in vitro (B). Reaction products were fractionated by SDS-polyacrylamide gel electrophoresis, and the dried gel was analyzed by phosphorimaging. Different levels of conjugation with SUMO-1 are indicated in A. We arbitrarily considered PK-PML conjugation as 100%. ++++, 100–67%; ++, 66–33%; and +, <33%. C, HeLa cells were cotransfected with plasmids encoding His6-SUMO-1 and NLS-PK or NLS-PK constructs encoding PML-(485–495), IκBα-(16–26), and p53-(381–391) amino acid sequences. Transfected cells were processed as in Fig. 3. Purified His6-SUMO-1 conjugates and protein input were detected by Western blotting using an anti-Myc antibody.

Regulation was determined in vitro. Whereas changing the Leu to either Ile or Val increased the efficiency of SUMO-1 conjugation, changes to Ala, Pro, or Trp substantially reduced the efficiency of SUMO-1 conjugation. Substitutions of Leu with Phe or Met decreased the efficiency of conjugation with SUMO-1 (4, 5), suggesting that the short sequence containing the Lys and Glu residues, the large hydrophobic residue contributes substantially to the efficiency of SUMO-1 modification. Short transferable sequences from various protein substrates modified with SUMO-1 show different capacities to be conjugated with SUMO-1. The best conjugated short sequence contains the sequence LKSE from RanGAP1 (381–391)–wild type (L), PK-PML-(485–495)–wild type (M), and indicated mutants were tested as substrates for SUMO-1 conjugation in vitro. Different levels of conjugation with SUMO-1 are indicated.

DISCUSSION

Post-translational protein modifications modulate protein function by altering protein activity or the ability to interact with ligands or by changing subcellular localization of the modified protein. Conjugation with SUMO-1 has been proposed to regulate protein function through all these mechanisms. Identification of a short amino acid sequence motif required for the transfer of the capacity to be conjugated with SUMO-1 to a heterologous protein indicates that this motif is necessary for recognition by the SUMO-1 modification enzymes. Most of the targets for SUMO-1 modification are Ubcb9-interacting proteins, and it is likely that substrate specificity is achieved by Ubcb9. The C-terminal region of Ubcb9, which is thought to be involved in substrate binding, lies close to the catalytic site and favors the direct transfer of SUMO-1 to substrate proteins (38). The most important amino acids in the consensus sequences are Lys and Glu. Whereas the acceptor Lys residue cannot be substituted, Glu can be replaced by Asp to generate a recognition motif that, although functional, is poorly conjugated with SUMO-1 (Fig. 4).

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Although nuclear targeting appears to be required for SUMO-1 modification of most substrates, we cannot rule out the possibility that some proteins may be modified in other cellular compartments. This may be the case for the glucose transporters GLUT1 and GLUT4, which are targeted to the cell membrane and yet appear to be SUMO-1-modified (42). One possibility is that newly synthesized SAE and Ubc9 are recruited to a cytoplasmic complex containing GLUT1 and GLUT4, where modification takes place. This is consistent with the observation that GLUT1 and GLUT4 both interact directly with Ubc9. If the NLSs of SAE and Ubc9 are occluded in this complex, then this would allow a small proportion of the predominantly nuclear SAE and Ubc9 to remain in the cytoplasm tightly associated with their substrate.

The two recently described SMT3-specific proteases Ulp1 and Ulp2 (11, 12) accumulate a different pattern of SMT3-conjugated proteins in their mutants, indicating that deconjugation of substrates can be achieved and regulated by multiple SMT3 proteases with different specificities. Because SUMO-1 is a limiting factor for conjugation of substrates, deconjugation of SUMO-1 may be a dual mechanism to decrease (or increase) protein activity of a deconjugated protein and increase (or decrease) the activity of a newly conjugated target, when released SUMO-1 is available. However the cellular sites at which this process takes place are not known, because the cellular localization of endogenous SUMO-1-specific proteases has yet to be determined. Thus SUMO-1 modification of most proteins appears to be regulated by the requirement of the substrate to be targeted to the nucleus and by the possession of a SUMO-1 recognition motif displayed on the surface of the target protein. It is likely that SUMO-1 modification emerges as an important control mechanism that regulates the activity of many nuclear proteins.

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REFERENCES

1. Johnson, P. R., and Hochstrasser, M. (1997) Trends Cell Biol. 7, 408–413
2. Saitoh, H., Pu, R. T., and Dasso, M. (1997) Trends Biochem. Sci. 22, 574–578
3. Hodges, M., Tissot, C., and Freemont, P. S. (1998) Curr. Biol. 8, R749–R752
4. Mahajan, R., Delphin, C., Guan, T., and Melchior, F. (1999) Cell 88, 97–107
5. Matonis, M. J., Coutavas, E., and Blobel, G. (1996) J. Cell Biol. 135, 1457–1470
6. Saitoh, H., Sparrow, D. B., Shiomi, T., Pu, R. T., Nishimoto, T., Matoh, T. J., and Dasso, M. (1998) Curr. Biol. 8, 121–124
7. See, M. L., Jensen, K., and Wei, R. H. J. (1997) J. Biol. Chem. 272, 1621–1634
8. Gostissa, M., Hengstermann, A., Fogal, V., Sandy, P., Schwarz, S. E., Scheffner, M., and Del Sal, G. (1999) EMBO J. 18, 6402–6411
9. Li, S. J., and Hochstrasser, M. (1999) Nature 398, 246–251
10. Li, S. J., and Hochstrasser, M. (2000) Mol. Cell 20, 2367–2377
11. Suzuki, T., Ichiyama, A., Saitoh, H., Kawakami, T., Omata, M., Chung, C. H., Kimura, M., Shimbara, N., and Tanaka, K. (1999) J. Biol. Chem. 274, 3131–3134
12. Gong, L., Millas, S., and Yeh, E. T. (2000) J. Biol. Chem. 275, 3355–3359
13. Kato, K. I., Baek, S. H., Jeon, Y. J., Nishimori, S., Suzuki, T., Uchida, S., Shimbara, N., Saitoh, H., Tanaka, K., and Chung, C. H. (2000) J. Biol. Chem. 275, 14102–14106
14. Johnson, E. S., Schwienhorst, I., Dobben, R. J., and Blobel, G. (1997) EMBO J. 16, 5509–5519
15. Desterro, J. M. P., Rodriguez, M. S., Kemp, G. D., and Hay, R. T. (1999) J. Biol. Chem. 274, 10618–10624
16. Gong, L., Li, B., Millas, S., and Yeh, E. T. (1999) FEBS Lett. 448, 185–189
17. Okuma, T., Honda, R., Ichikawa, G., Tsubomura, Y., and Yasuda, H. (1999) Biochem. Biophys. Res. Commun. 254, 693–698
18. Gong, L., Li, B., Millas, S., and Yeh, E. T. (1999) FEBS Lett. 448, 185–189
19. Okuma, T., Honda, R., Ichikawa, G., Tsubomura, Y., and Yasuda, H. (1999) Biochem. Biophys. Res. Commun. 254, 693–698
20. Desterro, J. M. P., Thomson, J., and Hay, R. T. (1997) FEBS Lett. 417, 297–300
21. Johnson, E. S., and Blobel, G. (1997) J. Biol. Chem. 272, 26989–26992
22. Schwarz, S. E., Matuschewski, K., Liakopoulos, D., Scheffner, M., and Jentsch, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 560–564
23. Lee, G. W., Melchior, F., Matonis, M. J., Mahajan, R., Tan, Q., and Anderson, D. F. (1998) J. Biol. Chem. 273, 6503–6507
24. Seufert, W., Butler, B., and Jentsch, S. (1995) Nature 373, 78–81
25. Arenzana-Seisdedos, F., Turpin, P., Rodrigues, M., Thomas, D., Hay, R. T.,...
Virelizier, J. L., and Dargemont, C. (1997) J. Cell Sci. 110, 369–378
26. Ossareh-Nazari, B., Bachelerie, F., and Dargemont, C. (1997) Science 278, 141–144
27. Rodriguez, M. S., Wright, J., Thompson, J., Thomas, D., Baleux, F., Virelizier, J. L., Hay, R. T., and Arenzana-Seisdedos, F. (1996) Oncogene 12, 2425–2435
28. Ossareh-Nazari, B., Bachelerie, F., and Dargemont, C. (1997) Science 278, 141–144
29. Rodriguez, M. S., Thompson, J., Hay, R. T., and Dargemont, C. (1999) J. Biol. Chem. 274, 9108–9115
30. Firestein, R., and Feuerstein, N. (1998) J. Biol. Chem. 273, 5892–5902
31. Duprez, E., Saurin, A. J., Desterro, J. M., Lallemand-Breitenbach, V., Howe, K., Boddy, M. N., Solomon, E., de The, H., Hay, R. T., and Freemont, P. S. (1999) J. Cell Sci. 112, 381–393
32. Johnson, E. S., and Blobel, G. (1999) J. Cell Biol. 147, 981–994
33. Sternsdorf, T., Jensen, K., Reich, B., and Will, H. (1999) J. Biol. Chem. 274, 12555–12566
34. Arenzana-Seisdedos, F., Thompson, J., Rodriguez, M. S., Bachelerie, F., Thomas, D., and Hay, R. T. (1995) Mol. Cell. Biol. 15, 2689–2696
35. Matunis, M. J., Wu, J. A., and Blobel, G. (1998) J. Cell Biol. 140, 499–509
36. Kamitani, T., Nguyen, H. P., and Yeh, E. T. H. (1997) J. Biol. Chem. 272, 14001–14004
37. Kamitani, T., Nguyen, H. P., Kito, K., Fukuda-Kamitani, T., and Yeh, E. T. H. (1998) J. Biol. Chem. 273, 3117–3120
38. Liu, Q., Jin, C., Liao, X., Shen, Z., Chen, D. J., and Chen, Y. (1999) J. Biol. Chem. 274, 16979–16987
39. Jiang, J., Zhang, Y., Krainer, A. R., and Xu, R. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3572–3577
40. Zhong, S., Muller, S., Ronchetti, S., Freemont, P. S., Dejean, A., and Pandolfi, P. P. (2000) Blood 95, 2748–2752
41. Ishov, A. M., Sotnikov, A. G., Negorev, D., Vladimirova, O. V., Neff, N., Kamitani, T., Yeh, E. T., Strauss, J. F., III, and Maul, G. G. (1999) J. Cell Biol. 147, 221–234
42. Giorgino, F., de Robertis, O., Laviola, L., Montrone, C., Perrini, S., McCowen, K. C., and Smith, R. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1125–1130
