Fission Yeast Homolog of Murine Int-6 Protein, Encoded by Mouse Mammary Tumor Virus Integration Site, Is Associated with the Conserved Core Subunits of Eukaryotic Translation Initiation Factor 3*

The murine int-6 locus, identified as a frequent integration site of mouse mammary tumor viruses, encodes the 48-kDa eIF3ε subunit of translation initiation factor eIF3. Previous studies indicated that the catalytically active core of budding yeast eIF3 consists of five subunits, all conserved in eukaryotes, but does not contain a protein closely related to eIF3ε/Int-6. Whereas the budding yeast genome does not encode a protein closely related to murine Int-6, fission yeast does encode an Int-6 ortholog, designated here Int6. We found that fission yeast Int6/eIF3ε is a cytoplasmic protein associated with 40S ribosomes. FLAG epitope-tagged Tif35, a putative core eIF3g subunit, copurified with Int6 and all five orthologs of core eIF3 subunits. An int6 deletion (int6Δ) mutant was viable but grew slowly in minimal medium. This slow growth phenotype was accompanied by a reduction in the amount of polyribosomes engaged in translation and was complemented by expression of human Int-6 protein. These findings support the idea that human and Schizosaccharomyces pombe Int-6 homologs are involved in translation. Interestingly, haploid int6Δ cells showed unequal nuclear partitioning, possibly because of a defect in tubulin function, and diploid int6Δ cells formed abnormal spores. We propose that Int6 is not an essential subunit of eIF3 but might be involved in regulating the activity of eIF3 for translation of specific mRNAs in S. pombe.

Mouse mammary tumor virus (MMTV)1 integrates into the mouse genome and frequently causes mammary tumors. The sites of MMTV integration were identified to delineate the molecular basis of the tumorigenesis (see Refs. 1 and 2 for review). Except for int-6, the int loci encode growth factors or transmembrane receptors. Thus, altered expression of these Int proteins leads to mammary tumorigenesis presumably by affecting signal transduction pathways controlling cell growth.

By contrast, the molecular basis for tumorigenesis caused by integration into int-6 is unclear. Int-6 encodes a ubiquitously expressed, 52-kDa protein, which corresponds to the 48-kDa eIF3ε subunit2 of eukaryotic translation initiation factor 3 (eIF3) (3). int-6 is the site of MMTV integration in at least three independently isolated mouse tumors; in all cases, MMTV integration into an intron of the int-6 gene resulted in expression of truncated int-6 mRNA species (4). Therefore, MMTV integration into int-6 may produce a dominant negative allele, because of expression of a truncated, unregulatable form of the protein. Alternatively, the truncation may simply disrupt the biological function of Int-6, and tumorigenesis would result from reduced int-6 gene dosage.

Although Int-6 is a stable component of mammalian eIF3, it is not known whether it is required for the activity of eIF3 in translation initiation (3). Furthermore, two laboratories (5–7) have localized a significant proportion of Int-6 in the nucleus, particularly in a nuclear compartment called the promyelocytic leukemia (PML) nuclear body (8). Nuclear localization of an Int-6 ortholog was also reported in Arabidopsis thaliana (9). These results raised the possibility that integration into int-6 affected the role of Int-6 in the nucleus but not in the cytoplasm as a component of eIF3. To identify the biological role of Int-6 and decipher the consequences of its truncation, it was important to establish the role of Int-6 in translation initiation as a part of eIF3. Thus far, eIF3 has been purified from mammalian cells (10, 11), wheat germ (12, 13), A. thaliana (14), and budding yeast Saccharomyces cerevisiae (15–17). Mammalian eIF3 associates with the 40S ribosome and stimulates the binding of both mRNA and Met-tRNAiMet (10, 11). Purified S. cerevisiae eIF3 was also shown to stimulate Met-tRNAiMet binding to 40S ribosomes in vitro (16, 17).

The eIF3 purified from budding yeast consists of five proteins, eIF3a (TIF32), eIF3b (PRT1), eIF3c (NIP1), eIF3g (TIF35), and eIF3i (TIF34), homologous to mammalian eIF3 subunits and does not contain an ortholog of human eIF3ε/Int-6 (17, 18). Moreover, the complete S. cerevisiae genome sequence does not encode a protein closely related to Int-6. Thus, an Int-6-related protein does not seem to be involved in the functions of eIF3 in the yeast Saccharomyces. Besides

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† The abbreviations used are: MMTV, mouse mammary tumor virus; eIF, eukaryotic translation initiation factor; PML, promyelocytic leukemia; ORF, open reading frame; GFP, green fluorescence protein; BSF, ribosomal salt wash; WCE, whole cell extracts; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; EMM, Edinburgh minimal medium; DAPI, 4’,6-diamidino-2-phenylindole.

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Fission Yeast Int6 is a Part of eIF3

**TABLE I**
List of oligonucleotides used in this study

| Sequence | Description | Tag
|----------|-------------|----
| 1 | GCCCATAGGGAGTCGGAGCTTAAAGG | 5’ end of int6‘ ORF | NdeI
| 2 | GCCCTCGCTAAGCTGCTCCGTCTTAA | 3’ end of int6‘ ORF | XhoI
| 3 | GCCCATAGGTATGATCAGTAATGTAAT | 5’ end of tif35‘ ORF | NdeI
| 4 | CGGAGACCTCGAGCTTGAAGATT | 3’ end of int6‘ ORF | BglII
| 5 | CCGGGATCTCAGATAAGCGCAATCTTGAAG | 3’ end of human int6 ORF | BamHI
| 6 | GCCGACTCTGAGGTGTTTATGCTGTTACCTCCACACCAACAGCACCTTTTTTTTTGCG | For int6‘ gene replacement |
| 7 | TGCATACAGTGCTCCGTCTTAAAC | For tif35‘ gene replacement |
| 8 | CGAATACGTTAAGTACAGTCA | For int6‘ gene replacement |

* Restriction enzyme recognition sites used for tagging the polymerase chain reaction fragments are italicized.

Int-6, mammalian eIF3 contains three other noncore subunits, eIF3δ (p66), eIF3β (p47), and eIF3h (p40), which are conserved in plants, Caenorhabditis elegans, and Drosophila melanogaster but absent in S. cerevisiae (19). Thus, it is possible that the activities of eIF3 from higher eukaryotes are modulated by association of the five core subunits with Int-6 and the other eIF3 subunits not found in S. cerevisiae. Interestingly, the recent progress in sequencing the entire genome of fission yeast Schizosaccharomyces pombe has revealed that it encodes the four orthologs of noncore eIF3 subunits, in addition to orthologs of the five core subunits (14). Thus, S. pombe eIF3 may resemble mammalian eIF3 in containing several polypeptides in addition to the five core subunits present in budding yeast.

In this study, we examined the role of the S. pombe protein, Int6, closely related to murine Int-6. We found that Int6 is predominantly localized in the cytoplasm, unlike mammalian and plant Int-6. We also provide evidence that Int6 is a stable component of fission yeast eIF3 and is functionally homologous to human Int-6 protein. Unlike tif35‘, which encodes an essential core eIF3g ortholog, int6‘ is dispensable. The int6Δ mutant grew slowly in minimal medium, however, and this slow growth phenotype was accompanied by reduction in the amount of polyriboosomes engaged in translation. Our results implicate Int-6 homologs in translation initiation and lay the groundwork for investigating the cytoplasmic function of Int-6 using fission yeast as a model organism.

MATERIALS AND METHODS

**Plasmid and Yeast Strains**—The DNA segment containing the int6‘ open reading frame (ORF) was amplified by polymerase chain reaction with oligonucleotides 1 and 2 (Table I) from genomic DNA, digested with NdeI and XhoI, and subcloned into the NdeI and SalI sites of pREP41 (20) or pREP41HA N (21), producing pREP-int6 or pRHA-int6, respectively. The tif35‘ ORF was similarly amplified with oligonucleotides 3 and 4, digested with NdeI and BglII, and subcloned into the NdeI and BamHI sites of pRF41, a derivative of pREP41 containing the coding sequence for the FLAG epitope, inserting it between the BamHI and SmaI sites of pRF41. The resultant pREP41-tif35-FLAG plasmid encodes eIF3g (Tif35) FLAG-tagged at its C terminus under the control of the nmt1 thiamine-repressible promoter.

The DNA segment encompassing the human int-6 ORF was amplified by polymerase chain reaction with oligonucleotides 5 and 6 (Table I) from human genomic DNA, digested with XhoI and BamHI, and subcloned into the BamHI site in pREP41-tif35-FLAG in a plasmid containing the FLAG epitope at its C terminus under the control of the nmt1 thiamine-repressible promoter. The DNA fragment was used to replace the S. pombe int6‘ ORF in pRHA-int6 to generate pRHA-hInt-6.

**Purification of the Putative eIF3 Complex Containing FLAG-Tif35/ Int35**—Transformants of JW350 (int6‘-3HA) carrying pREP-tif35-FLAG, or pREP41 as a control, were grown in 2 liters of EMM-leu-B1 to A600nm ~ 10 and harvested by centrifugation. After washing with sterile water, cells (~ 10 g) were suspended in 15 ml of buffer A (25) and homogenized with a French Press (Spectronic Unicam). After clearing the extract by two rounds of centrifugation (13,000 × g for 20 min), the supernatant was subjected to centrifugation at 39,000 rpm for 4.5 h, and separated into twenty 0.6-ml fractions. A portion of the first fifteen fractions from the resulting ribosomal pellet was subjected to in gel trypsin digestion and matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry at Integraproteomics, Inc. (Toronto, Canada). The observed mass spectrum of its tryptic peptides was compared with the predicted spectra of all the proteins in the data base using the ProFound program.

**RESULTS**

The Phenotypes of S. pombe Cells Lacking the int6‘ Gene—Fission yeast S. pombe encodes a hypothetical protein (GenBank accession number CAA22813) homologous to murine eIF3e/Int6. This protein, designated Int-6 here, is 45% identical and 57% similar to murine Int6. Because this extent of similarity is only slightly less than that between murine Int-6 and related proteins in C. elegans (52% identical) and...
melanogaster (62% identical), we suspected that Int6 is the S. pombe homolog of mammalian Int-6.

First we examined the effect on cell growth of deleting the int6+ gene. We transformed a diploid S. pombe strain with an int6 deletion construct containing a selectable kanamycin resistance gene (kan) in place of the int6+ ORF and confirmed the deletion of one allele of chromosomal int6+ by Southern blot analysis. When the resulting heterozygous diploid strain was subjected to sporulation on synthetic sporulation agar and stained with DAPI, the asterisk indicates an example of displaced nuclei, and the arrowheads indicate examples of unequal nuclear partitioning in septated cells. C, the int6Δ cells were subjected to sporulation on synthetic sporulation agar and stained with DAPI. The asterisk indicates a binucleate fused spore, and the arrowhead indicates an abnormally shaped spore.

Next, we examined the intracellular localization of Int6. For this purpose, we constructed a haploid yeast strain (JW346) encoding the int6 protein fused to GFP, in place of the wild type int6 protein (see “Materials and Methods”). JW346 grew indistinguishably from its isogenic wild type parent (JY450) both in rich and minimal media (data not shown), indicating that GFP-Int6 is functional. As shown in Fig. 2, fluorescence was observed throughout the cytoplasm and was absent from the nucleus. Therefore, Int6 is predominantly localized in the cytoplasm.

Int6 Is Part of a High Molecular Mass Complex That Associates with 40S Ribosomes—Because eIF3 associates with 40S ribosomes, it was important to examine whether Int6 is associated with 40S ribosomes in S. pombe cells. We constructed strain JW350 containing an HA epitope-tagged allele (int6–3HA) in place of the chromosomal wild type allele. HA-Int6 is functional, because JW350 grew indistinguishably from the wild type both in rich and minimal media (data not shown). As a control, we analyzed the S. cerevisiae strain KAY50 encoding an HA-tagged form of eIF3i subunit TIF34 (HA-TIF34), because it was shown that eIF3 is associated with 40S subunits in budding yeast (27). Cell extracts prepared from these strains in the presence of cycloheximide were resolved by sucrose gradient velocity sedimentation, and the resulting absorbance profiles indicated that the ribosomal species in S. pombe cosedimented exactly with the corresponding ribosomal species in S. cerevisiae (Fig. 3, A and B, upper panels). Immunoblot analysis of the gradient fractions showed that the majority of HA-Int6 sediments with the bulk of 40S subunits in S. pombe extracts, consistent with its physical association with 43–48S preinitiation complexes (Fig. 3A, lanes 7 and 8). As expected, a large fraction (~50%) of the HA-TIF34 in S. cerevisiae extracts sedimented at exactly the same location (Fig. 3B, lanes 7 and 8).

We also found that a subpopulation of both HA-Int6 (Fig. 3A, lanes 4 and 5) and HA-TIF34 (Fig. 3B, lanes 4 and 5) sediments in fractions just preceding the 40S ribosome, suggesting that these proteins are present in high molecular mass complexes. Immunoblotting of the S. cerevisiae fractions with antibodies against the budding yeast eIF3b (PRT1) supports the notion that the high molecular mass complex containing HA-TIF34 at fractions 4–5 is the eIF3 complex of ~600 kDa (17, 18, 28). As expected, the S. cerevisiae eIF2 complex of 124 kDa sediments in fractions closer to the top of the gradient, with a peak in fraction 3, whereas the bulk of eIF1 (13 kDa) peaked in fraction 2 (Fig. 3B, bottom two panels). Thus, the sedimentation pattern of HA-Int6 strongly suggests that Int6 is associated with a high molecular mass complex, most likely...
Int6 localizes in the cytoplasm. Cellular localization of Int6 was examined using the int6Δ::GFP strain JW348. Cells proliferating in YES medium were fixed by cold methanol and stained with DAPI. Fluorescence of GFP (B) represents Int6 localization, whereas DAPI staining (A) indicates the position of nuclei. C is the superimposition of the image of A in red with the image of B in green.

FIG. 3. Int6 is associated with 40 S ribosomes and a high molecular-mass complex that cosediments with S. cerevisiae eIF3. Twenty A260 units of WCEs prepared from S. pombe strain JW350 (int6Δ::3HA) (A) and S. cerevisiae strain KAY50 (TIF34-HA) (B) were resolved by velocity sedimentation on 15–40% sucrose gradients and then analyzed by SDS-PAGE (data not shown). Because functional eIF3 complexes purified from S. cerevisiae contained only five core subunits eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i (16, 17), we suspected that the five major polypeptides, eIF3a, eIF3b, eIF3c, and eIF3i, in addition to (FL-) Tif35/eIF3g itself (see the Introduction). To test this idea directly, we processed each of the five polypeptides for mass spectrometry (see “Materials and Methods”), and we identified all five S. pombe orthologs of S. cerevisiae core eIF3 subunits and the heat shock protein Hsp70 (Table II). Coomassie staining of the purified complex eluted from the resin revealed seven major polypeptides (p105, p85, p70, p40, p39, p32, and p20) and several other minor constituents (p64, p60, p46, p43, and p36). None of these proteins were contained in the fraction prepared from a control untagged tie35Δ strain (lane 1). Immunoblotting of the purified complex revealed that the 60-kDa minor constituent (p60) reacted with anti-HA antibodies (lanes 3 and 4), whereas p39, p32, and p20 reacted with anti-FLAG antibodies (lanes 5 and 6). These results suggest that p60 and p39 are Int6-HA and FL-Tif35, respectively, and that p32 and p20 are degradation products of FL-Tif35. Identification of p60 as HA-Int6 was supported by preparing the FL-Tif35 complex from a control strain that carries pREP-tif35-FLAG but contains untagged int6Δ and finding a 58-kDa protein instead of p60 in SDS-PAGE (data not shown).

Because functional eIF3 complexes purified from S. cerevisiae contained only five core subunits eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i (16, 17), we suspected that the five major polypeptides in the FL-Tif35-containing complex, p105, p85, p70, p40, and p39 would correspond to the predicted S. pombe proteins, eIF3a, eIF3b, eIF3c, and eIF3i, in addition to (FL-) Tif35/eIF3g itself (see the Introduction). To test this idea directly, we processed each of the five polypeptides for mass spectrometry (see “Materials and Methods”), and we identified all five S. pombe orthologs of S. cerevisiae core eIF3 subunits and the heat shock protein Hsp70 (Table II). Coomassie staining of the purified complex eluted from the resin revealed seven major polypeptides (p105, p85, p70, p40, p39, p32, and p20) and several other minor constituents (p64, p60, p46, p43, and p36). None of these proteins were contained in the fraction prepared from a control untagged tie35Δ strain (lane 1). Immunoblotting of the purified complex revealed that the 60-kDa minor constituent (p60) reacted with anti-HA antibodies (lanes 3 and 4), whereas p39, p32, and p20 reacted with anti-FLAG antibodies (lanes 5 and 6). These results suggest that p60 and p39 are Int6-HA and FL-Tif35, respectively, and that p32 and p20 are degradation products of FL-Tif35. Identification of p60 as HA-Int6 was supported by preparing the FL-Tif35 complex from a control strain that carries pREP-tif35-FLAG but contains untagged int6Δ and finding a 58-kDa protein instead of p60 in SDS-PAGE (data not shown).

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4. B. Cox, personal communication.
stand the high salt buffer used to strip initiation factors from the ribosomes in preparing the RSW. The fact that the amount of Int6 was substoichiometric compared with the amounts of the five core subunits, including FL-Tif35, may indicate that only a portion of eIF3 contains Int6. However, it is possible that Int6 is a stoichiometric component of eIF3 but was partially removed from the core complex during the RSW preparation. Based on the results shown in Figs. 3 and 4 and Table II, we propose that Int6 is physically associated with at least a portion of the eIF3 complexes in S. pombe.

Deletion of int6 Diminishes Translation Initiation in Minimal Medium—As shown in Fig. 1, deletion of int6 was not lethal and, instead, showed pleiotropic phenotypes including slow growth in EMM and defects in mitosis and meiosis. To determine whether the int6Δ slow growth phenotype is associated with reduced rate of translation initiation, we analyzed the polysome profiles in cell extracts prepared from strains JW346 (int6Δ) and JY450 (int6+) grown in thiamine-depleted EMM for 16 h to induce FLAG-Tif35 expression from pREP41-tif35-FLAG. Cell extracts were prepared and subjected to immunoprecipitation (CoIP) with an anti-FLAG antibody (panel 1) or anti-HA antibody (panel II). Immunoprecipitates were separated by SDS-PAGE and examined for the presence of the indicated proteins by immunoblotting using anti-FLAG or anti-HA antibodies (lanes 4–6). One-fourth of the total crude extracts were separated and analyzed similarly (lanes 1–3). B, FL-Tif35 complex purified from the RSW contains HA-Int6. The FLAG-Tif35 complex (+, lanes 2, 4, and 6) and the control eluate prepared in parallel from a strain carrying the vector pREP41 (−, lanes 1, 3, and 5) were prepared as described under “Experimental Procedures.” A portion of these samples was subjected to SDS-PAGE, followed by Coomassie staining (lanes 1 and 2) or immunoblotting with anti-HA or anti-FLAG antibodies (lanes 3–6). Proteins specifically associated with FL-Tif35 in addition to HA-Int6 were labeled by their sizes in kDa.

**Fig. 4. Int6 resides in a multisubunit complex with the S. pombe ortholog of S. cerevisiae eIF3g subunit Tif35.** A, Int6 forms a complex in vivo with the S. pombe ortholog of S. cerevisiae TIF35. The transformants of int6–3HA strain JW350 carrying the empty vector pREP41 (lanes 1 and 4) or pREP41-tif35-FLAG (lanes 2 and 5) and the transformant of JY450 (int6+) carrying pREP41-tif35-FLAG (lanes 3 and 6) were grown in thiamine-depleted EMM for 16 h to induce FLAG-Tif35 expression from pREP41-tif35-FLAG. Cell extracts were prepared and subjected to immunoprecipitation (CoIP) with an anti-FLAG antibody (panel 1) or anti-HA antibody (panel II). Immunoprecipitates were separated by SDS-PAGE and examined for the presence of the indicated proteins by immunoblotting using anti-FLAG or anti-HA antibodies (lanes 4–6). One-fourth of the total crude extracts were separated and analyzed similarly (lanes 1–3). B, FL-Tif35 complex purified from the RSW contains HA-Int6. The FLAG-Tif35 complex (+, lanes 2, 4, and 6) and the control eluate prepared in parallel from a strain carrying the vector pREP41 (−, lanes 1, 3, and 5) were prepared as described under “Experimental Procedures.” A portion of these samples was subjected to SDS-PAGE, followed by Coomassie staining (lanes 1 and 2) or immunoblotting with anti-HA or anti-FLAG antibodies (lanes 3–6). Proteins specifically associated with FL-Tif35 in addition to HA-Int6 were labeled by their sizes in kDa.
phenotype of int6. As shown in Fig. 6, the S. pombe encoding HA-tagged S. pombe respiratory deficiency did not alter the previously that a slow growth rate in budding yeast caused by mutation. As controls, we used the empty vector pREP41HA N, moter and investigated whether this plasmid could comple- HA-tagged human Int-6 under the thiamine-repressible pro- resulted in cell cycle arrest primarily in the G1 phase, although eIF3 bound to 40 S ribosomes. of which (at least) eIF3i (34) and Tif35/eIF3g are essential. However, no other effects were observed in cells carrying the vector or the vector encoding Int6. Cells expressing Int6 showed several phenotypes, including hypersensitivity to the microtubule-depolymerizing drug, thia- Responding to these observations, we examined whether human Int-6 could substitute at least partially for the function of S. pombe Int6. Because the slow growth phenotype of int6Δ cells is associated with a reduced rate of translation initiation, we evaluated the translation initiation of int6Δ cells (Fig. 5). Thus, Int6 is functionally homologous to human eIF3d subunit p66, which is also indispensable for normal translation in mammalian cells. Our results suggest that human Int-6 protein can function in place of S. pombe Int6 in translation initiation.

**DISCUSSION**

In this study, we characterized a fission yeast protein Int6 closely related to murine Int-6 protein, encoded by a frequent MMTV integration site. We showed that Int6 is cytoplasmic (Fig. 2) and associates with ribosomes in vivo (Fig. 3). Affinity purification of the putative eIF3 complex of S. pombe directed against FLAG epitope-tagged Ti635/eIF3g revealed the presence of Int6 and the five proteins homologous to the core subunits of budding yeast eIF3 (Fig. 4 and Table II). This is the first evidence that fission yeast eIF3 contains the five core proteins that comprise budding yeast eIF3 (17, 18) and also occur in mammalian eIF3 (19). In addition to the five core subunit orthologs, we also identified the fission yeast ortholog of eIF3g (PIR accession number T04090, 33 kDa) as the 36-kDa polypeptide in the FLAG-Tif35 complex shown in Fig. 4B (data not shown). Thus, physical interactions between the core and noncore subunits appear to be conserved between the fission yeast and mammalian eIF3 complexes. Furthermore, the complementation of the int6Δ slow growth phenotype by human int-6 suggests that S. pombe Int6 is functionally homologous to mammalian Int-6 (Fig. 6).

Despite the evidence for physical interaction between Int-6 and the putative core eIF3 subunits, int6+ is not an essential gene, and we observed only a moderate reduction in the rate of protein synthesis initiation in int6Δ cells (Fig. 5). Thus, Int6 is dispensable for the essential activities of eIF3. moe1+ encoding the S. pombe homolog of human eIF3d subunit p66, is also nonessential (33), although its association with S. pombe eIF3 remains to be determined. Presumably, the essential functions of S. pombe eIF3 depend only on the five core subunit orthologs, of which (at least) eIF3Δ (34) and Tif35/eIF3g are essential.

We found that int6Δ cells showed several phenotypes, including frequent unequal nuclear partitioning during mitosis in haploids, abnormal spore formation in diploids (Fig. 1), and hypersensitivity to the microtubule-depolymerizing drug, thia-bendazole. If Int6 is involved in general translation initiation, how can deletion of int6+ lead to such specific phenotypes? One possible explanation is that a moderate reduction in eIF3 function caused by int6Δ severely impaired translation of a subset of mRNAs with relatively poor initiation regions that compete poorly for ribosomes and other factors with mRNAs containing optimal initiation sites. Those mRNAs whose translation was strongly reduced in int6Δ cells would include mRNAs encoding proteins critical for nuclear partitioning or sporulation. An alternative explanation for the pleiotropic int6Δ phenotype is that Int6 is involved in gene-specific translational control, e.g., by facilitating recruitment of a specific subset of mRNAs to the eIF3 bound to 40 S ribosomes.

In S. cerevisiae, reduced protein synthesis rates caused by mutations in eIF3 subunit genes TIF34, PRT1, or TIF32/RPG1 result in cell cycle arrest primarily in the G1 phase, although the tif34 mutation additionally impedes the G2/M transition (29, 35, 36). In contrast, int6Δ has more profound effects on the completion of mitosis (Fig. 1B) than on the start of the cell cycle, in favor of the model that Int6 is involved specifically in translational control in G2. In keeping with this idea, deletion of moe1+ encoding another noncore eIF3 subunit homolog impairs microtubule function and confers resistance to microtubule-destabilizing reagents (35), suggesting a link between Moe1 (eIF3d) and Int6 (eIF3e) in mitosis. Recently, one of the mis (minichromosome instability) mutations in fission yeast was shown to impair 18 S ribosomal RNA biogenesis, suggesting that a decrease in general protein synthesis rates would impair chromosome segregation (37). Understanding the molecular basis for mitotic defects caused by disruption of the translation machinery will require further examination.

While this manuscript was under review, two articles were previously published reporting the characterization of the Int-6 ortholog in S. pombe (38, 39). Crane et al. (38) proposed that S. pombe Int-6 is a part of eIF3, based on coimmunoprecipitation of epitope-tagged eIF3i and eIF3b orthologs with Int-6-HA. We demonstrated this point by affinity purification (Fig. 4B) and subsequent immunoblot (Fig. 4B) and mass spectrometry (Table II) analyses of a FLAG-Tif35/eIF3g-containing complex. This purified complex contained seven of the nine eIF3 subunit proteins: eIF3a, eIF3b, eIF3c, eIF3d, eIF3f, eIF3g, and eIF3i. Additionally, the presence of eIF3j was also confirmed by immunoblotting with an eIF3j-specific antibody. This result is consistent with the recent findings of de Boer et al. (39) that eIF3j is required for normal translation in S. pombe.
orthologs encoded in fission yeast. We also showed that the amount of polysomes engaged in protein synthesis was reduced significantly in the int6Δ mutant (Fig. 5). These results are consistent with the polysome analysis of Bandyopadhyay et al. (39), who also observed a moderately reduced rate of total protein synthesis in the int6Δ mutant, as measured by amino acid incorporation. Together, these data provide convincing biochemical evidence that Int6 enhances the rate of translation initiation in vivo. These articles also reported interesting phenomena associated with overexpression and deletion of int6Δ. Overexpression of int6Δ conferred multi-drug resistance, dependent on the role of Int6 in activating multidrug resistance the PML nuclear bodies where Int-6 resides, has been implicated in tumors of certain genes. PML, protein the major constituent of in vivo. This suggests that disruption of the nuclear function of Int-6 hence, the frequency of tumorigenesis (40). However, it remains possible that overexpression, nor did it complement the nuclear sensitivity of int6Δ cells. In contrast, the nuclear partitioning defect we observed in int6Δ cells (Fig. 1B) was complemented by expression of human Int-6 protein. Thus, the role of Int-6 in mitosis may be conserved between fission organisms will contribute greatly to solving this complicated problem.

Acknowledgments—We are greatly indebted to Henry Levin for critical reading of the manuscript and for sharing materials used for experiments on fission yeast. We thank Yoshiya Kubo for disruption of int6Δ haploids also happens frequently for mice carrying the MMTV genome inserted at one allele of int-6, this might increase the degree of aneuploidy and, hence, the frequency of tumorigenesis (40). However, it remains possible that disruption of the nuclear function of Int-6 is responsible for tumorigenesis by altering the transcription rates of certain genes. PML, protein the major constituent of the PML nuclear bodies where Int-6 resides, has been implicated as a tumor suppressor and transcriptional regulator (8). Although fission yeast Int6 was not localized in the nucleus (Fig. 2), the role of Int6 in activating multidrug resistance appears to be independent of the role of Int6 in translation (38). Understanding the role of Int-6 by using S. pombe as a model organism will contribute greatly to solving this complicated problem.

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