A meiotic DNA polymerase from a mushroom, *Agaricus bisporus*

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A meiotic DNA polymerase [DNA nucleotidyltransferase (DNA-directed), EC 2.7.7.7], which likely has a role in meiotic DNA repair, was isolated from a mushroom, *Agaricus bisporus*. The purified fraction displays three bands in SDS/PAGE, at molecular masses of 72 kDa, 65 kDa and 36 kDa. Optimal activity is at pH 7.0–8.0 in the presence of 5 mM Mg^{2+} and 50 mM KCl and at 28–30 °C, which is the temperature for meiosis. This enzyme is resistant to *N*-ethylmaleimide and sensitive to 2′,3′-dideoxythymidine 5′-triphosphate, suggesting that it is a β-like DNA polymerase. These characteristics are similar to those of *Coprinus* DNA polymerase β [Sakaguchi and Lu (1982) Mol. Cell. Biol. **2**, 752–757]. In Western-blot analysis, the antiserum against the *Coprinus* polymerase reacts only with the 65 kDa band, which coincides with the molecular mass of the *Coprinus* polymerase. Western-blot analysis also showed that the antiserum could react with crude extracts not only from the *Agaricales* family, to which *Agaricus* and *Coprinus* belong, but also from different mushroom families and *Saccharomyces*. The *Agaricus* polymerase activity can be found only in the meiotic-cell-rich fraction, but the enzyme is also present in the somatic cells in an inactive state.

INTRODUCTION

The β class of eukaryotic DNA polymerases has been extensively characterized in a variety of animals, particularly in many phyla of the major phylogenetic branch termed the deuterostomia, which includes mammals (Wang, 1991). In the 1980s, organisms such as the protostomic animals (Sakaguchi and Boyd, 1985), plants (Sakaguchi et al., 1980), fungi (Sakaguchi and Lu, 1982) or protozoa (Furukawa et al., 1979) were also reported to have β-like polymerase activity, implying that the enzyme is universally present in eukaryotes. These lower organisms had once been thought to lack polymerase β. The basis of this early misconception is the fact that 'lower' organisms do not necessarily have the enzyme in every tissue or at every developmental stage (see Sakaguchi et al., 1980; Sakaguchi and Lu, 1982).

Polymerase β, as far as deuterostomic animals are concerned, has been implicated in several aspects of DNA metabolism (Wang, 1991), although its precise role *in vivo* still remains to be determined. As some have proposed, its role may be functionally related to somatic DNA repair (Perrino and Loeb, 1990), which includes the replacement synthesis of DNA (Hanawalt et al., 1979). *Lilium* (Sakaguchi et al., 1980) and *Coprinus* (Sakaguchi and Lu, 1982) produce β-like polymerase only during meiotic prophase. Three factors taken together lend credence to the possibility that, in these organisms, a β-like polymerase plays a role in meiotic recombination. A form of replacement synthesis occurs during meiotic prophase (Sakaguchi and Lu, 1982). Precisely that stage of meiosis associated with replacement synthesis is correlated with the appearance of β-like polymerase. Furthermore, that stage correlates with a lack of any other polymerases besides polymerase β. These earlier reports reveal a potentially versatile role for polymerase β, but that role has yet to be thoroughly defined (Boyd et al., 1989).

Organisms which have a β-like polymerase only in meiosis are of particular interest to researchers seeking a more precise definition of the polymerase β function in eukaryotic DNA repair and recombination. From this point of view, we have long studied meiotic DNA polymerase from meiocytes in a higher plant, *Lilium*, or a mushroom, *Coprinus cinereus* (Sakaguchi and Lu, 1982). Unfortunately, because the cells at that stage are so few, this approach has thus far yielded little definitive information about the enzyme. Another finding based on the *Coprinus* data needs to be confirmed and systematically explored. This is the finding that the meiotic polymerase may also function as a reverse transcriptase, because not only poly(C)·$(dG)_{12-18}$ but also a native viral RNA can become a good template. However, the lack of sufficient meiotic polymerase for study has hampered this line of research.

To secure a reliable source, we have screened tissues to find ones that are suitably meiosis-rich from which the polymerase can be purified. We ultimately chose caps at the meiotic stage of an edible mushroom, *Agaricus bisporus*, which can provide a reliable source. At present, we have succeeded in the mass isolation of the polymerase from the edible mushroom. This enzyme was very similar to a meiotic polymerase from a distantly related mushroom, *Coprinus* genus, as reported previously (Sakaguchi and Lu, 1982). Western-blot analysis suggested that the structure may be widely conserved in primitive eukaryotes, suggesting that polymerase β has an exclusive evolutionary role in meiotic DNA repair which accompanies chromosome recombination.

MATERIALS AND METHODS

Materials

Nucleotides, calf thymus and salmon sperm DNA, and chemically synthetic template-primers such as poly(A), poly(C), $(dT)_{12-18}$ or $(dG)_{12-18}$ were purchased from Sigma. [^{3}H]TTP (43 Ci/mmol) and [^{3}H]dGTP (25–50 Ci/mmol) were purchased from New England Nuclear Corp. DNA polymerase inhibitors such as 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP) and *N*-ethylmaleimide (NEM) were obtained from Pharmacia LKB and Wako Chemicals respectively. DEAE-cellulose and phosphocellulose P11 were purchased from Serva and Whatman, and

Abbreviations used: ddTTP, 2′,3′-dideoxythymidine 5′-triphosphate; NEM, *N*-ethylmaleimide; ssDNA, single-stranded DNA; PMSF, phenylmethanesulphonyl fluoride.

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single-stranded DNA (ssDNA)–cellulose was made in our laboratory by the method of Alberts et al. (1968). Calf thymus DNA was used for ssDNA cellulose. The Mono Q HR5/5 column (f.p.l.c. system) was purchased from Pharmacia LKB. All other reagents were of analytical grade and were purchased from Wako Chemicals.

Collection of fruiting caps at meiotic stages

By observing the mushroom karyogamy as a cytological marker of meiotic prophase (Lu, 1969), *Agaricus* meiotic caps were collected in Fuji Biofarm Inc. (Japan) and then dissected in our laboratory to cut out the meiosis-rich tissue. More than two-thirds of the tissue was at the meiotic prophase. *Flammulina velutipes*, *Lentinus edodes* and *Glifola frondosa* caps were obtained from other mushroom farms, and the same procedures were followed. The tissues were quickly frozen in liquid nitrogen and stored before use at −80 °C.

Enzyme assay

The method for analysing DNA polymerase activity was basically the same as described previously (Sakaguchi and Lu, 1982). The salmon sperm DNA template-primer was activated with pancreatic DNAase by the method of Schlabach et al. (1971). The assay mixture (0.2 ml) contained: 50 mM Tris/HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 40 mM each of dATP, dCTP and dGTP, unlabelled 0.04 mM TTP, 5 μCi of [^{3}H]TTP/ml, 200 mg of activated DNA/ml, 15% glycerol and 0.05 ml of enzyme solution. The reaction mixture was incubated at 25 °C in a water bath, and the reaction was stopped by addition of 1 ml of 0.1 M sodium pyrophosphate on ice. After that, 0.5 mg of BSA was added as a carrier before 1 ml of 20% trichloroacetic acid in 0.1 M sodium pyrophosphate was added to precipitate the acid-insoluble product. The mixture was filtered on glass-fibre filters (GF/A; Whatman), and acid-insoluble radioactivity was measured in a Beckman scintillator. One unit of DNA polymerase activity is defined as the amount of enzyme required to convert 1 pmol of dTMP into acid-insoluble material in 1 h at 25 °C.

Extraction and purification of the enzyme

The frozen *Agaricus* cap tissues (about 10–30 g wet wt.) were ground with a mortar and a pestle at 4 °C in 50 ml of 0.05 M Tris/HCl (pH 7.5)/1 mM EDTA/5 mM 2-mercaptoethanol containing protease inhibitors [1 μg/ml each of leupeptin and pepstatin A, and 1 mM phenylmethanesulphonyl fluoride (PMSF)]/40% glycerol (TEMG) containing 0.6 M NaCl. The homogenate was completely disrupted in a French press, and then 150 ml of TEMG buffer containing 0.6 M NaCl was added. After being centrifuged for 20 min at 15000 *g* and 4 °C, the supernatant was diluted 3-fold with TEMG buffer without NaCl. The diluted fraction was loaded on a phosphocellulose column (5.0 cm diam. × 4.0 cm) equilibrated with 0.04 M phosphate buffer containing 1 mM EDTA, 5 mM 2-mercaptoethanol and 40% glycerol (PEMG), and then the active fraction, after being washed with 0.2 M PEMG, was collected with 0.6 M PEMG. The active fraction was dialysed against TEMG buffer, loaded on a DEAE-cellulose column (5.0 cm diam. × 6.0 cm) equilibrated with 0.2 M KCl in TEMG buffer and then eluted with a linear gradient of 0.2–0.5 M KCl in TEMG. The active fractions were pooled and dialysed against TEMG buffer. The dialysed fraction was loaded on a ssDNA–cellulose column (2.5 cm × 3.0 cm) equilibrated with TEMG buffer. The flow-through fraction, in which DNA polymerase activity was observed, was loaded on a Mono Q HR5/5 column equilibrated with TEMG buffer and then eluted with a linear gradient of 0–1.0 M NaCl in TEMG (see Figure 2). The active fractions were pooled, dialysed against TEMG buffer and stored as a solution in 50% glycerol at −20 °C.

Isoelectric-focusing analysis

The methods employed were essentially those developed by LKB. Electrophoresis was performed in a 110 ml gradient of 5–40% sucrose containing 2% (v/v) carrier ampholyte (pH range 3.5–10), 5% glycerol, 0.1% Triton X-100, 1 μg/ml each of leupeptin and pepstatin A, 1 mM PMSF and the enzyme fraction. The gradients were run for 48 h at 4 °C and then collected in 2 ml fractions.

SDS/PAGE

SDS/PAGE was conducted as described by Laemmli (1970) with a 12.5%-acrylamide running gel. After electrophoresis, the gel was treated with silver stain as described by Wray et al. (1981).

Production of antiserum

The antiserum used in this work was made against β-type DNA polymerase from *Coprinus*, which is distantly related to *Agaricus*. Three 8-week-old rats were used for producing the antiserum by immunizing with the purified DNA polymerase. There was no immunological cross-reactivity between non-immune serum and the DNA polymerase. Primary immunization was made subcutaneously with 0.15 mg (in 0.5 ml) of the polymerase preparation suspended in 0.5 ml of Freund's complete adjuvant. Booster immunizations consisting of the same amount of the polymerase in the same adjuvant were given twice at 1-week intervals, and, after 10 days from the last booster injection, the serum was collected by normal preparation methods (Cooper et al., 1990).

Western-blot analysis

Western-blot analysis was performed by the modified methods described in Current Protocols in Molecular Biology (Winston et al., 1990). The crude extracts were separated by SDS/PAGE and then electrophoretically transferred to nitrocellulose filters in transfer buffer (20 mM Tris, 150 mM glycine, pH 8.0, 20% methanol). Nitrocellulose filters were blocked in 4% skimmed-milk powder in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4,7H_2O$, 1.4 mM KH_2PO_4). The screening antibody was diluted in 4% skimmed-milk powder in PBS and incubated with the filters. The filters were washed with PBS, incubated with conjugated anti-rat IgG, washed with PBS and then made visible by adding a precipitable substrate solution (15 mg of 4-chloro-1-naphthol, 5 ml of methanol, 25 ml of PBS, 10 ml of 30% H_2O_2).

Preliminary enzyme fractionation

The *Agaricus* cap tissues from zygotene–pachytene cells and tetrad cells were ground, disrupted, and centrifuged as described above (see under 'Extraction and purification of the enzyme'). The supernatants were made up to 55% saturation with $(NH_4)_2SO_4$ and allowed to settle overnight at 4 °C. The solutions were centrifuged for 10 min at 12000 *g* and the pellets were suspended with TEMG buffer. The salt concentration of the

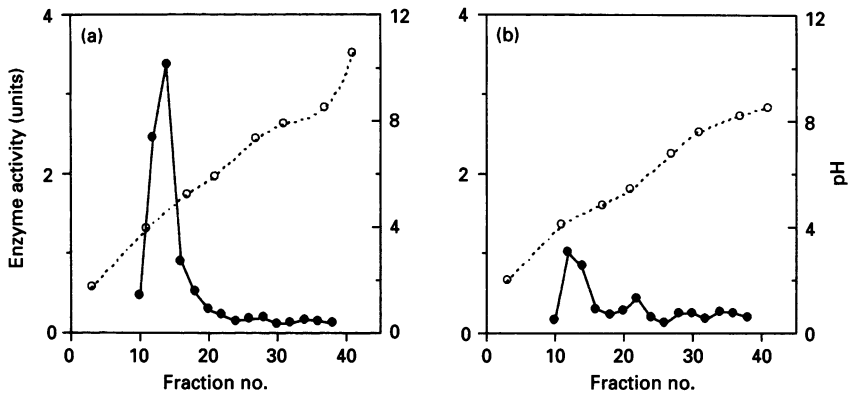

Figure 1 Isoelectric focusing of DNA polymerase activity from different stages of *Agaricus bisporus*

Extracts were subjected to electrophoresis on a 110 ml LKB Ampholine column as described in the Materials and methods section from zygotene–pachytene-stage cells (**a**) and from tetrad-stage cells (**b**). ●, Polymerase activity; ○, pH.

suspension was lowered by dialysis against TEMG buffer in preparation for isoelectric focusing.

RESULTS AND DISCUSSION

Our main concern has been to determine whether β-like DNA polymerase is present in meiotic cells in *Agaricus bisporus* and whether this commercialized vegetable can provide a reliable source of the enzyme for precise analysis of the polymerase β function.

Preliminary experiments showed that mushroom caps of size about 0.8–1.5 cm radius were mostly at meiotic prophase stages and that the caps sold at the food markets were mainly at the spore-formation stages after meiosis. *Agaricus* caps in meiosis were collected at a mushroom farm, where they were cultivated on a large scale for food. We could therefore easily obtain abundant amounts of *Agaricus* meiotic caps.

In *Agaricus*, a preliminary survey of meiotic DNA polymerase was performed by fractionating crude extracts by isoelectric focusing. The mushroom meiotic tissues at a zygotene–pachytene stage or a tetrad stage were disrupted, and the crude enzyme fractions were prepared by the methods described in the Materials and methods section. The fractions were directly electrophoresed in an isoelectrofocusing column (Figure 1): as shown, only one peak of DNA polymerase activity, with a pI value of approx. 5, occurred at the zygotene–pachytene stage (Figure 1a). This activity was much decreased at the tetrad stage which signals the end of meiosis (Figure 1b). None of the somatic tissues, such as the stalks or the mycelium, exhibit any such activity at all, but they showed an α-like polymerase activity (Sakaguchi and Lu, 1982). The remainder of the present paper is devoted to an analysis of the pI 5 enzyme as a meiotic DNA polymerase.

Purification was the next step, and was performed by the methods described in the Materials and methods section. In an attempt to optimize the recovery and avoid a partial degradation of the enzyme structure, several precautions were taken at the extraction and purification stages of the process.

The most serious problem is proteolysis. Therefore, not only did the extraction buffer contain three protease inhibitors, leupeptin, pepstatin A and PMSF, but also purification had to be completed within 2 h during all steps until the proteases were flushed by 0.2 M phosphate PEMG through the phosphocellulose. To save time, the steps for dialysis and concentration were omitted. The crude extracts containing high salt were directly diluted with TEMG after being disrupted and centrifuged. The diluted extracts bound to the phosphocellulose after flushing were collected with 0.6 M phosphate PEMG. Almost all the protease activity could be separated in this step.

The fraction was next applied to a DEAE-cellulose column. One major and two minor activities appeared on elution with 0.35 M, 0.4 M and 0.45 M KCl respectively. The major one was the target for the next column step (denatured DNA–cellulose); the polymerase did not bind to the denatured DNA cellulose. SDS/PAGE showed that the fraction was still separated into several polypeptides. The flow-through fraction, in which DNA polymerase activity was observed, was loaded on a Mono Q column. The polymerase activity was retained and eluted at 0.7 M KCl (Figure 2a). Arrows in Figures 2(a) and 2(b) indicate the fraction with the greatest activity. Figure 2(b) depicts the SDS/PAGE of every active fraction from the Mono Q chromatography. Each fraction around the active area has been separated into three polypeptides by SDS/PAGE, indicating molecular masses of 72 kDa, 65 kDa and 36 kDa. The 65 kDa polypeptide coincided with the pattern of polymerase activity, and the lane with the densest band of the 72 kDa or 36 kDa polypeptide appeared to be slightly shifted left from the activity peak top. As described below, the 65 kDa polypeptide was actually the meiotic DNA polymerase. Since the peak fraction was thought to be pure enough to characterize, it was used as the *Agaricus* meiotic DNA polymerase fraction in this report. The other two polypeptides may be impurities. For example, DNA ligase II is a polypeptide in the size range 68–72 kDa (Lindahl and Barnes, 1992). The tight association between both enzymes may be possible in the meiotic DNA metabolism. The 36 kDa polypeptide seemed to resemble closely a meiotic DNA-binding protein which enhances only the meiotic DNA polymerase activity (Gomi and Sakaguchi, 1994). Such studies are in progress and will be reported elsewhere.

Sepharose 6B gel filtration of the *Agaricus* meiotic polymerase fraction was done both with and without high salt (0.5 M KCl). Even in the presence of 0.5 M KCl, the molecular mass was around 820 kDa. In PAGE without SDS, no proteins in the *Agaricus* meiotic polymerase fraction could enter the gel.

Mg^{2+} at 5 mM and univalent-ionic strength of 50 mM KCl were required for optimum activity of the polymerase. Mg^{2+} was essential. Optimum activity is exhibited in a broad range of pH,

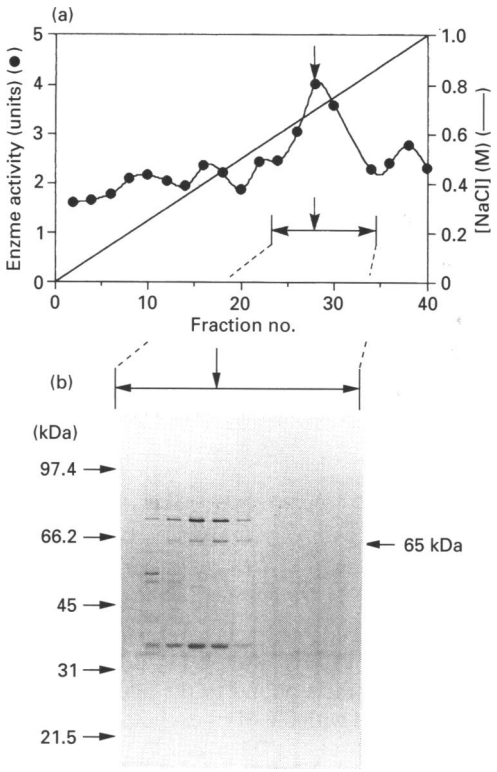

Figure 2 Mono Q column chromatography and SDS/PAGE of meiotic DNA polymerase

The active fraction from ssDNA–cellulose column chromatography was applied on a Mono Q column (**a**), and each active fraction from Mono Q column chromatography was electrophoresed in a 12.5%-polyacrylamide slab gel with SDS under the condition described in the Materials and methods section (**b**). Markers were rabbit muscle phosphorylase *b* (97.4 kDa), BSA (66.2 kDa), hen egg-white ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa) and soybean trypsin inhibitor (21.5 kDa).

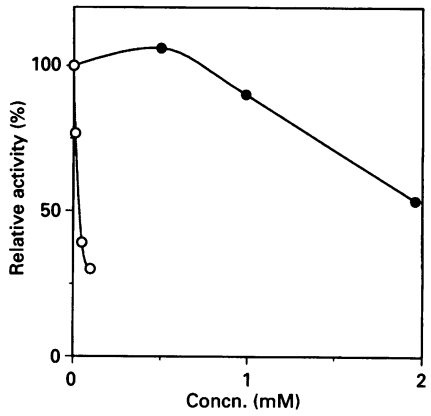

Figure 3 Inhibitor effects on meiotic DNA polymerase activity

Polymerase assay conditions were as described in the Materials and methods section, except for adding the indicated concentration of inhibitors: ●, NEM; ○, ddTTP.

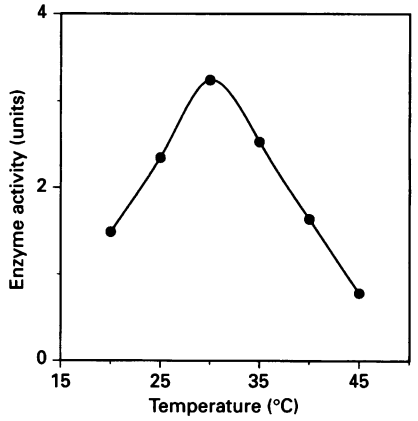

Figure 4 Effect of temperature on the activity of *Agaricus* DNA polymerase

Polymerase assay conditions were as described in the Materials and methods section, except for the indicated temperature. The incubation time was 1 h.

7.0–8.0. The polymerase activity was resistant to NEM but sensitive to ddTTP, indicating that it is a β-type DNA polymerase, according to the international nomenclature established by Burgers et al. (1990) (Figure 3).

Optimum activity was achieved at 30 °C, and the activity was decreased to 70 and 75% at 25 and 35 °C respectively (Figure 4). The data concerning the optimum temperature is quite interesting, because the polymerase from *Agaricus* mycelium has an optimum at 37 °C (results not shown). In most mushroom species, vegetative growth of the mycelium is efficient at 35–37 °C, but fruiting bodies are formed at 25–30 °C. If formed at 35 °C, the bodies would have abnormal meiosis and consequently nuclearless spores, or would die. In general, meiosis in any eukaryote is a process that requires a temperature suitable for meiotic DNA metabolism. The low optimum temperature may indicate that the polymerase is readily adapted in the meiotic cell-cycle machinery. Since β-type polymerase appears to be universally present in eukaryotic meiocytes as a result of their evolution (Sakaguchi and Lu, 1982), and since the enzyme tends to be optimized at lower temperature (as shown here and previously; Sakaguchi and Lu, 1982), DNA polymerase β may originate from meiotic DNA polymerase.

A special polymerase response to template-primers should be emphasized. Although all types of DNA polymerase β reported can utilize a synthetic RNA homopolymer, poly(A)·$(dT)_{12-18}$, as a template-primer, the *Agaricus* meiotic polymerase is an exception, which uses poly(C)·$(dG)_{12-18}$ instead of poly(A)·$(dT)_{12-18}$. As is widely recognized, the poly(C)-type template is only for a RNA-dependent DNA polymerase (reverse transcriptase) from single-stranded RNA virus. This *Agaricus* meiotic polymerase should be investigated to see if it can reversely transcribe native single-stranded RNA. The further characterization of the RNA dependency is in progress. The *Agaricus* meiotic polymerase appears to be quite similar to the *Coprinus* polymerase.

In order to investigate further, we performed a Western-blot analysis using rat antiserum made against the purified *Coprinus* β-type DNA polymerase, which was composed of a 65 kDa polypeptide (Matsuda et al., 1993). The analysis showed only one band in SDS/PAGE of the *Agaricus* crude extracts (lane 2 in Figure 5). The band corresponds to the 65 kDa polypeptide from the *Agaricus* meiotic polymerase fraction. This antiserum also displayed a band with a molecular mass of 65 kDa, which exactly corresponds to the *Coprinus* polymerase in the *Coprinus* crude extracts (lane 1 in Figure 5). Since the antiserum can partially precipitate the polymerase activities from both *Agaricus* and

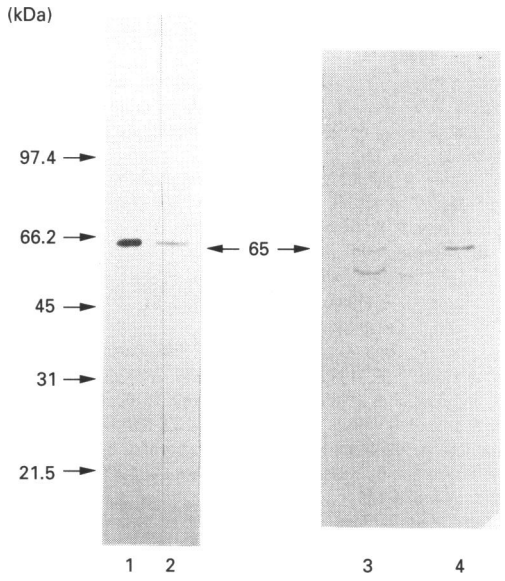

Figure 5 Western-blot analysis of meiotic DNA polymerase

The crude extract electrophoresed in a 12.5%-polyacrylamide gel was blotted on a nitrocellulose filter and made to react with the antiserum against *Coprinus* meiotic polymerase as described in the Materials and methods section. Lanes: 1, crude extract from *Coprinus* caps; 2, crude extract from *Agaricus* caps; 3, crude extract from *Agaricus* caps left overnight at room temperature; 4, crude extract from *Agaricus* stalk.

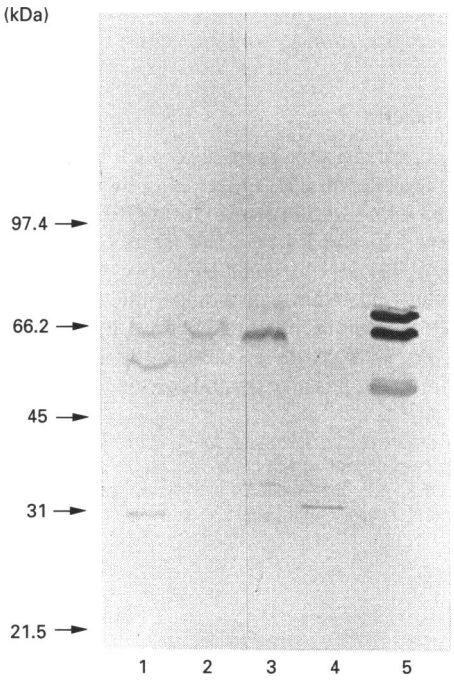

Figure 6 Immunological comparison among various fungi with *Agaricus* DNA polymerase

Western-blot analysis conditions and procedure were the same as described in the legend of Figure 5. Crude extracts from *Agaricus* (lane 1), *Flammulina* (lane 2), *Lentinus* (lane 3), *Grifola* (lane 4) and *Saccharomyces* (lane 5).

Coprinus, the 65 kDa polypeptide in *Agaricus* may be a core protein with DNA polymerase activity, and the enzyme structure appears to be very similar to, or almost identical with, the polymerase in *Coprinus*, which is a distant relative of *Agaricus*.

An unexpected piece of information in the Western-blot analysis was that a crude extract from the *Agaricus* fruiting-body's stalks, which consist of somatic cells, displayed a band of molecular mass 65 kDa (lane 4 in Figure 5). This fact was confirmed in the mycelium. As stated above, β-type DNA polymerase activity has never been found in somatic cells of any of the eukaryotes except animals, but only in meiotic cells. An explanation for lane 4 in Figure 5 is that the inactive form may be present irrespective of the type of cells. Since the molecular masses of the enzyme from both the meiotic and somatic cells were obviously the same, the somatic 65 kDa polypeptide seems not to be the precursor.

When the crude extract of the meiocytes is left overnight at room temperature, the meiotic 66 kDa polypeptide is degraded. The molecule was separated into two polypeptides with molecular masses of 65 kDa and 56 kDa (lane 3 in Figure 5), and then 65 kDa, 56 kDa and 31 kDa polypeptides (lane 1 in Figure 6). Interestingly, the somatic 65 kDa polypeptide has never been cleaved under the same conditions. Whether the decrease in size is meaningful for the activation or the result of degradation is unknown.

Apart from the mechanism of polymerase production during meiosis, there were intriguing questions concerning the universality of polypeptides and their decrease in size, which suggested the need for employing a comparative methodology. We decided to check for these polypeptides in other, distantly related, mushrooms and in a yeast (Figure 6). If these polypeptides were universally found in the Western blot, the size decrease might suggest a process like activation. The 65 kDa polypeptide was observed in mushroom caps at early meiosis-rich stages of *Flammulina* and *Lentinus*, which are mushrooms in the *Agaricales* family to which *Agaricus* and *Coprinus* also belong. The 65 kDa polypeptide, namely a meiotic DNA polymerase, appears to exist generally in *Agaricales* (Figure 6). Moreover, if the idea of size decrease could be adapted to the other polypeptides, the blotted bands would be observed in a different mushroom family, *Grifola*, and even in *Saccharomyces* (Figure 6). These smaller polypeptides may be the enzyme core, which may be widely preserved in primitive eukaryotes or the fungus kingdom, including the basidiomycetes. According to Shimizu et al. (1993), the DNA polymerase β of *Saccharomyces cerevisiae* was a 68 kDa polypeptide. In Figure 6 under the special conditions, the largest band was at 68 kDa, and two more polypeptides of decreased size (65 kDa and 50 kDa) were observed.

The role *in vivo* of DNA polymerase β, compared with other polymerases, involves a question which must still be thoroughly defined. A few animal species, including mammals, are reported to have β-activity in all somatic cells, and the enzyme has been a centre for studying the role. These species are generally higher organisms on the animal phylogenetic tree. Although it is designated as a meiotic polymerase (Sakaguchi et al., 1980; Sakaguchi and Lu, 1982), because polymerase β has been universally found only in the meiotic cells in our present and past studies, and because the somatic tissue in most of the species has no such activity, its anomalous appearance in some somatic cells of some higher animals cannot be ignored. This somatic polymerase might have originated as a meiotic enzyme which had a role only in meiotic DNA repair or recombination. In later evolution, the enzyme then might be spread throughout the

whole body after acquiring a possible role in relation to DNA repair or recombination in somatic cells.

Concluding remarks

We have demonstrated the presence of meiotic DNA polymerase in *Agaricus bisporus*, and its universality in the other mushroom families. According to Stern and Hotta (1974) and our previous report (Sakaguchi and Lu, 1982), a meiotic prophase of *Lilium* and *Coprinus* has DNA repair synthesis. Our present results, indicating the universality of polypeptides and size decrease, suggest that the meiotic polymerase must be strongly related to meiotic repair DNA synthesis, namely the meiotic chromosome recombination, and has an essential role in genetic crossing-over.

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