# The catabolic function of the α-aminoadipic acid pathway in plants is associated with unidirectional activity of lysine–oxoglutarate reductase, but not saccharopine dehydrogenase

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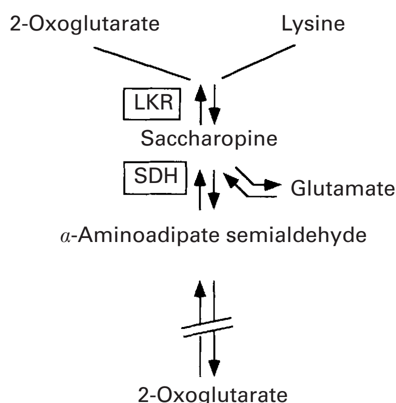
Whereas plants and animals use the α-aminoadipic acid pathway to catabolize lysine, yeast and fungi use the very same pathway to synthesize lysine. These two groups of organisms also possess structurally distinct forms of two enzymes in this pathway, namely lysine–oxoglutarate reductase (lysine–ketoglutarate reductase; LKR) and saccharopine dehydrogenase (SDH): in plants and animals these enzymes are linked on to a single bifunctional polypeptide, while in yeast and fungi they exist as separate entities. In addition, yeast LKR and SDH possess bi-directional activities, and their anabolic function is regulated by complex transcriptional and post-transcriptional controls, which apparently ascertain differential accumulation of intermediate metabolites; in plants, the regulation of the catabolic function of these two enzymes is not known. To elucidate the regulation of the catabolic function of plant bifunctional LKR/SDH enzymes, we have used yeast as an expression system to test whether a plant LKR/SDH also possesses bi-directional LKR and SDH activities, similar to the yeast enzymes. The *Arabidopsis* enzyme complemented a yeast SDH, but not LKR, null mutant. Identical results were obtained when deletion mutants encoding only the LKR or SDH domains of this bifunctional polypeptide were expressed individually in the yeast cells. Moreover, activity assays showed that the *Arabidopsis* LKR possessed catabolic, but not anabolic, activity, and its uni-directional activity stems from its structure rather than its linkage to SDH. Our results suggest that the uni-directional activity of LKR plays an important role in regulating the catabolic function of the α-amino adipic acid pathway in plants.

Key words: catabolism, essential amino acids, metabolism.

## INTRODUCTION

In contrast with the evolutionarily conserved nature of most metabolic pathways, the α-aminoadipic acid pathway functions differently in various groups of organisms. While yeast and fungi use this pathway to synthesize lysine (see Scheme 1), animals and plants use this very same pathway to catabolize lysine [1–3]. These two groups of organisms also possess structurally distinct forms of lysine–oxoglutarate reductase (lysine–ketoglutarate reductase; LKR) and saccharopine dehydrogenase (SDH), two central enzymes in the α-aminoadipic acid pathway. In plant and animal species these enzymes are linked on to a single bifunctional enzyme [2,4–8], whereas in yeast and fungi they reside on different polypeptides [9]. In addition, animal and plant LKR and SDH enzymes show much greater identity with each other than with their yeast and fungi counterparts, suggesting two distinct evolutionary routes of these enzymes that function either catabolically (in higher eukaryotes) or anabolically (in lower eukaryotes).




**Scheme 1 Schematic diagram of the α-amino adipic acid pathway**

Bi-directional activities of the various enzymes were derived from studies performed in yeast cells. The interrupted arrow represents seven enzymic reactions leading to the synthesis of 2-oxoglutarate (α-ketoglutarate).

The control of the anabolic function of the α-aminoadipic acid pathway in yeast has been largely elucidated. This control does not depend on intrinsic properties of LKR and SDH, because both of these yeast enzymes can catalyse the bi-directional conversion of their respective substrates: LKR mediates the conversion between lysine plus 2-oxoglutarate and saccharopine, and SDH mediates that between saccharopine and α-aminoadipic semialdehyde [1] (Scheme 1). Rather, the anabolic direction of the α-aminoadipic acid pathway in yeast is due to special transcriptional and post-transcriptional mechanisms controlling the expression of genes encoding enzymes of this pathway and, hence, production of favoured concentrations of intermediate metabolites for the anabolic activities of SDH and LKR [1,10,11].

In contrast with yeast, the mechanism controlling the catabolic function of LKR and SDH in plant and animal species is still not

Abbreviations used: LKR, lysine–oxoglutarate reductase (lysine–ketoglutarate reductase); SDH, saccharopine dehydrogenase; the prefixes At and Sc denote the enzymes from plant (*Arabidopsis thaliana*) and yeast (*Saccharomyces cerevisiae*) respectively.

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known. In the present report, we studied whether plant (*Arabidopsis thaliana*) LKR and SDH are capable of bi-directional conversion of their substrates, similarly to their yeast counterparts. We found that while the plant SDH possess a bi-directional activity and is able to complement a yeast SDH null mutant, the plant LKR possesses only a uni-directional catabolic activity and cannot complement a yeast LKR null mutant. Our results suggest that LKR plays an important role in the catabolic function of the $\alpha$-aminoadipic acid pathway in plants.

## EXPERIMENTAL

### Materials

Yeast strains 8973b (ura3, lys1) and 8989c (ura3, lys9) were kindly provided by A. Pierard (Laboratoire de Microbiologie, Faculte des Sciences, Universite Librede Bruxelles, Brussels, Belgium). Antibodies against an *Arabidopsis* LKR/SDH N-terminal peptide (AETVNKWERRTPLTPLTPSHC) were kindly provided by D. Miron (The Weizmann Institute of Science, Rehovot, Israel). Anti-(yeast LKR) polyclonal antibodies were kindly provided by A. Stepansky (The Weizmann Institute of Science, Rehovot, Israel). Antibodies against yeast cytochrome *c* oxidase were kindly provided by G. Schatz (University of Basel, Basel, Switzerland). Anti-His monoclonal antibodies were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.).

### Plasmid construction

The full-length *Arabidopsis* LKR/SDH cDNA (AtLKR/SDH) was originally cloned into the Bluescript (SK −) plasmid [8]. For construction of a chimaeric gene encoding AtLKR/SDHp fused at its N-terminus to a tag of six histidine residues (His tag), a double-stranded oligonucleotide comprising the two oligonucleotides 5′ CTAGAATGCACCACCACCACCACCACATG 3′ and 3′ TTACGTGGTGGTGGTGGTGGTGTACTTAA 5′ was used to replace the *Xba*I–*Eco*RI fragment of SK-AtLKR/SDH. The resulting clone was designated SK-AtHis-LKR/SDH.

The *Arabidopsis* monofunctional LKR cDNA clone (AtLKR) was produced by PCR to introduce a stop codon at the end of the LKR coding sequence. The yeast (*Saccharomyces cerevisiae*) LKR gene (*ScLKR*) was obtained by PCR of a yeast genomic DNA and subcloned into Bluescript. To add a histidine tag at the N-terminus of the yeast LKR, the PCR product was digested with *Eco*RI, and replaced the *Eco*RI–*Eco*RV DNA fragment of Sk-AtHis-LKR/SDH to generate Sk-ScHis-LKR. Yeast LKR lacking the SRL peroxisome targeting signal, or *Arabidopsis* LKR containing this signal, were obtained by PCR and then subcloned into Bluescript. All constructs in Bluescript were subcloned into the yeast expression vector pVT-102u [12].

### Yeast transformation and complementation

Expression plasmids were transformed into yeast lys1 and lys9 mutant cells using lithium acetate, as described previously [13]. For LKR and SDH complementation, positive transformants from lys1 and lys9 mutant cells harbouring the various constructs were plated on a synthetic complete (SC) lysine-free medium.

### SDS/PAGE and Western blot analysis

Protein extraction from yeast cells, fractionation on SDS/PAGE [14], transfer to PVDF membranes, staining of membranes with Coomassie Blue R and Western blot analysis were performed essentially as described previously [15], using an enhanced chemiluminescence immunodetection kit (Pierce).

### Purification of the recombinant LKR/SDH and monofunctional LKR enzymes

Partial purification of recombinant LKR/SDH and LKR enzymes was performed as described previously [16], with several modifications. Frozen yeast cells (2 g) were homogenized in 8 ml of 25 mM potassium phosphate buffer, pH 7.5, containing 1 mM EDTA, 5 % (v/v) glycerol and 1 mM PMSF. The extracts were then incubated with 4 ml of 80 % (v/v) CL-6B Sepharose (Pharmacia) at 4 °C for 30 min and centrifuged for 5 min at 18000 ***g***. The supernatant was incubated at 4 °C for 30 min with 1 ml of a 50 % slurry of nickel/nitrilotriacetate–agarose (Qiagen) in nickel/nitrilotriacetate gel buffer [25 mM potassium phosphate buffer, pH 7.5, 20 mM imidazole, 10 mM 2-mercaptoethanol and 5 % (v/v) glycerol], and centrifuged at 2000 ***g*** for 0.5 min. The column resin was washed with 20 ml of 25 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 5 % (v/v) glycerol, 300 mM NaCl and 10 mM 2-mercaptoethanol, resuspended in 5 ml of Mops buffer [50 mM Mops, pH 7.5, 1 mM EDTA and 5 % (v/v) glycerol], and transferred to a 5 ml column. The column was washed with 5 ml of Mops buffer, and LKR was eluted with 4 ml of Mops buffer containing 200 mM imidazole. Column fractions with maximal LKR activity were pooled.

### Protein determination and analysis of LKR activity

Protein levels were determined by the method of Bradford [17]. Anabolic LKR activity assays were performed essentially as described previously [18]. The activity assays included 0.1 M phosphate buffer (pH values between 6 and 7), 0.1 M Tris/HCl buffer (pH values between 7.5 and 9) or 0.1 M glycine buffer (pH 9.5), as well as 0.1 $\mu$g of purified proteins, 2 mM saccharopine and 2 mM $NAD^+$. The kinetics of LKR anabolic activity were measured spectrophotometrically by determining the change in $NAD^+$ reduction at 340 nm at 30 °C over 10 min. Each reaction also included a control lacking the substrate saccharopine. One unit of LKR anabolic activity was defined as the amount of enzyme needed to catalyse the reduction of 1 nmol of $NAD^+$ per min at 30 °C, using a value of 6.22 $mM^{-1} \cdot cm^{-1}$ as the millimolar absorption coefficient for NADH. Catabolic LKR activity was measured as described previously [19].

### Subcellular fractionation

Growth of yeast cells in peroxisome induction buffer and preparation of yeast spheroplasts were as described previously [20]. Spheroplasts were homogenized in sorbitol buffer (25 mM Mes/KOH, pH 6.0, 0.6 M sorbitol, 1 mM PMSF, 0.5 mM EDTA, 10 $\mu$g/ml leupeptin, 10 $\mu$g/ml aprotinin and 10 $\mu$g/ml pepstatin). A 1 ml portion of the resulting post-nuclear supernatant was layered over an 11 ml continuous (15–50 %, w/v) sucrose gradient. The gradient was centrifuged for 5 h at 4 °C at 250000 ***g*** in a Beckman SW40Ti rotor.

## RESULTS

### *Arabidopsis* LKR/SDH complements a yeast SDH-deficient mutant, but not an LKR-deficient mutant

Since both LKR and SDH in yeast can operate bi-directionally [1] (Scheme 1), the anabolic function of the $\alpha$-aminoadipic acid pathway in this organism is independent of the basic biochemical properties of these enzymes. To study the regulatory role of LKR and SDH in the catabolic function of this pathway in plants, we tested whether these enzymes can also operate bi-directionally, similar to their yeast counterparts. To this end, we have expressed the *Arabidopsis* bifunctional LKR/SDH enzyme in the yeast

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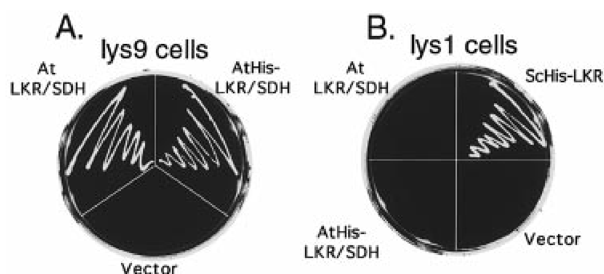


**Figure 1 Complementation of yeast lys1 and lys9 mutants by the *Arabidopsis* bifunctional LKR/SDH**

Yeast mutants lys9 (**A**), lacking SDH activity, and lys1 (**B**), lacking LKR activity, expressing AtLKR/SDH and AtHis-LKR/SDH were plated on lysine-free medium. Mutant cells expressing ScHis-LKR or the vector alone were used in parallel as positive and negative controls respectively. Positive complementation is shown by extensive cell growth along the streaking lines.

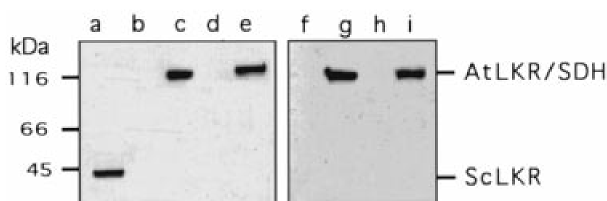


**Figure 2 Expression of the yeast LKR and *Arabidopsis* bifunctional LKR/SDH constructs in yeast lys1 and lys9 mutants**

Proteins were detected by Western blot analysis, using either anti-His-tag antibodies (lanes a–e) or antibodies against the *Arabidopsis* LKR/SDH N-terminal peptide (lanes f–i). Lane a, lys1 cells expressing ScHis-LKR; lane b, control lys1 cells expressing the vector alone; lane c, lys1 cells expressing AtHis-LKR/SDH; lane d, control lys9 cells expressing the vector alone; lane e, lys9 cells expressing AtHis-LKR/SDH; lane f, control lys1 cells expressing the vector alone; lane g, lys1 cells expressing AtLKR/SDH; lane h, control lys9 cells expressing the vector alone; lane i, lys9 cells expressing AtLKR/SDH. The molecular masses (kDa) of protein size markers are indicated on the left.

mutant lys1, lacking LKR activity, and in the mutant lys9, lacking SDH activity. Two *Arabidopsis* LKR/SDH constructs were used: one encoding a wild-type AtLKR/SDHp, and a second encoding the same protein fused at its N-terminus to six His residues (His tag) to facilitate the purification of the enzyme (AtLKR/SDH-HISp). To test whether AtLKR/SDHp and AtLKR/SDH-HISp could complement the two yeast mutants, transgenic lys1 and lys9 cells expressing the recombinant constructs encoding these proteins were grown in lysine-deficient media. As shown in Figure 1, both AtLKR/SDHp and AtLKR/SDH-HISp polypeptides complemented the yeast lys9 mutant, but not the lys1 mutant. The failure to complement lys1 was not due to impurity of this mutant, since a construct encoding a yeast ScLKRp complemented this mutant (Figure 1). Control yeast lys1 and lys9 cells harbouring the vector alone did not grow on lysine-deficient medium (Figure 1).

Next we tested whether the lack of complementation of the yeast lys1 mutant by AtLKR/SDHp stemmed from defective expression of this bifunctional polypeptide. Thus, after growing the transgenic yeast lines described above in lysine-containing media, soluble proteins were treated in a Western blot with either anti-His-tag antibodies or antibodies raised against a peptide derived from the N-terminus of the LKR domain of AtLKR/SDHp. As shown in Figure 2, cross-reacting bands of the size

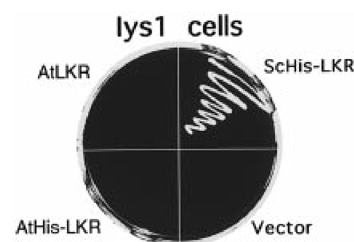


**Figure 3 Complementation of a yeast lys1 mutant by the *Arabidopsis* monofunctional LKR**

Yeast lys1 mutant cells expressing either AtLKR or AtHis-LKR were plated on lysine-free medium. Mutant cells expressing ScHis-LKR and the vector alone were used in parallel as positive and negative controls respectively. Positive complementation is shown by extensive cell growth along the streaking lines.

expected for AtLKR/SDHp were detected in extracts from the transgenic yeast lys1 and lys9 strains harbouring the AtLKR/SDH constructs, both with (Figure 2, lanes c and e) and without (Figure 2, lanes g and i) the His tag. These bands were not detected in control lys1 and lys9 cells harbouring the vector alone (Figure 2, lanes b, d, f and h). A cross-reacting band of the size expected for yeast LKR was detected in yeast cells expressing the recombinant His-tagged yeast LKR (Figure 2, lane a).

We then tested whether the enhanced anabolic SDH activity resulting from overexpression of AtLKR/SDHp in yeast brought about improved lysine production. Indeed, transformation of AtLKR/SDH into wild-type yeast cells resulted in a nearly 5-fold increase in free lysine levels compared with yeast cells transformed with the vector alone (results not shown).

### A dissected LKR domain of AtLKR/SDHp possesses efficient catabolic (but not anabolic) activity, and fails to complement the yeast lys1 mutant

The reason for the inability of AtLKR/SDHp to complement the yeast lys1 mutant is not clear. Unfortunately, it is impossible to test for the anabolic LKR activity of AtLKR/SDH because it cannot be resolved from the catabolic activity of the linked SDH domain of this bifunctional enzyme (both activities use the same substrates, saccharopine and $NAD^+$). However, AtLKR/SDHp expressed in yeast possessed efficient catabolic LKR activity (results not shown), suggesting that the LKR domain of AtLKR/SDHp was folded correctly. To explore further whether the lack of complementation of lys1 mutant cells was due to the structural conformation of the LKR domain or to its linkage to SDH, we expressed in these cells a deletion mutant of *Arabidopsis* LKR/SDH possessing only the LKR domain, with or without a His tag. The yeast lys1 cells transformed with these two versions of AtLKR were grown on lysine-free medium. As shown in Figures 3 and 4, neither version of AtLKRp could complement the yeast lys1 mutant, despite normal expression of both forms of AtLKRp.

To test whether AtLKRp produced in the yeast cells possessed anabolic LKR activity, the His-tagged AtLKRp, as well as ScLKRp as a control, were purified from the transgenic yeast cells using a nickel column, and tested for anabolic LKR activity at different pH values, ranging from 6 to 9.5. As shown in Figure 5, while the yeast ScLKRp possessed efficient anabolic activity, with a maximum at pH 9.5, the His-tagged AtLKRp did not possess any anabolic activity at any of the pH values tested. No anabolic activity of AtLKRp was detected even in incubation assays containing 5 mM hydrazine, a chelator of 2-oxoglutarate,

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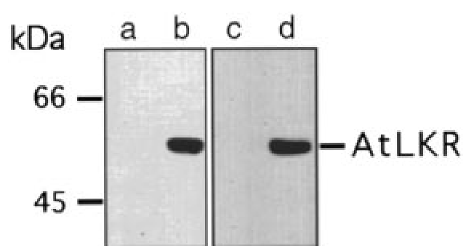


**Figure 4 Expression of *Arabidopsis* monofunctional LKR in yeast lys1 mutants**

Proteins were detected by Western blot analysis, using either anti-His-tag antibodies (lanes a and b) or antibodies against the LKR/SDH N-terminal peptide (lanes c and d). Lanes a and c, control lys1 cells expressing the vector alone; lane b, lys1 cells expressing AtHis-LKR; lane d, lys1 cells expressing AtLKR. The molecular masses (kDa) of protein size markers are indicated on the left.

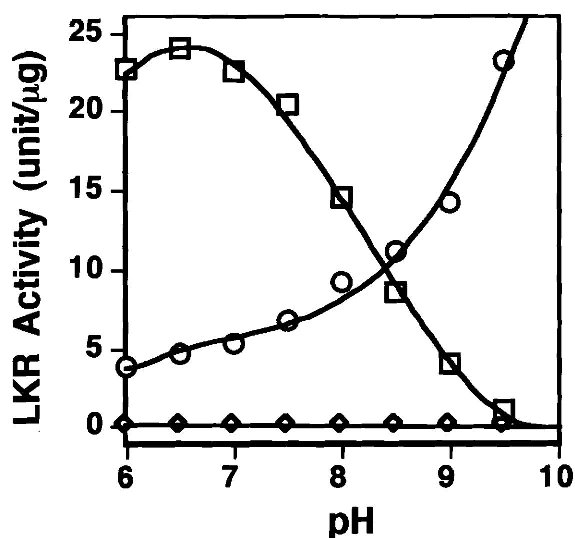


**Figure 5 Analysis of the anabolic and catabolic activities of the *Arabidopsis* monofunctional LKR expressed in yeast lys1 cells**

Purified His-tagged *Arabidopsis* monofunctional LKR was assayed for anabolic (◇) and catabolic (□) LKR activity at different pH values, as described in the Experimental section. The anabolic activity of purified His-tagged yeast LKR at the different pH values was measured as a positive control (○). The assays were repeated three times with similar results.

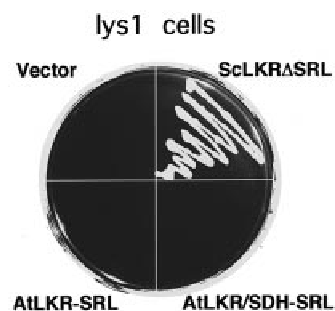


**Figure 6 The C-terminal SRL sequence of yeast LKR has no functional role in its anabolic activity *in vivo***

To test whether the C-terminal SRL sequence plays any functional role, yeast lys1 cells were transformed with recombinant constructs encoding yeast LKR lacking this signal (ScLKR Δ SRL), or with *Arabidopsis* LKR/SDH or monofunctional LKR possessing this signal. Yeast lys1 cells possessing a vector alone were used as a negative control. Positive complementation was scored by extensive cell growth along the streaking lines.

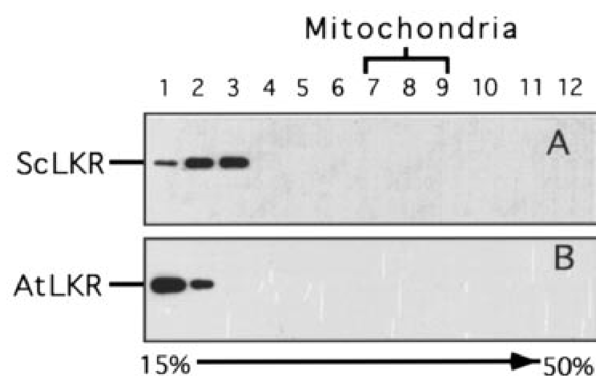


**Figure 7 Subcellular fractionation of yeast and *Arabidopsis* LKR expressed in yeast cells**

Post-nuclear supernatants prepared from cells expressing the yeast LKR (**A**) or the *Arabidopsis* LKR (**B**) were fractionated on a continuous 15–50% (w/v) sucrose gradient. Proteins from individual fractions were subjected to Western blot analysis, using either anti-(yeast LKR) antibodies (**A**) or antibodies against the *Arabidopsis* LKR/SDH N-terminal peptide (**B**). Fractions 1–12 are indicated. The migration of the mitochondrial marker enzyme cytochrome *c* oxidase is also indicated ('Mitochondria').

thus further confirming that the lack of such activity was not because of 'product inhibition' by 2-oxoglutarate (results not shown). Also, the lack of anabolic activity could not be accounted for by misfolding, because the same His-tagged AtLKRp possessed efficient catabolic activity, with maximum around pH values of 6.5–7 (Figure 5). Similar pH maxima were reported previously for LKR activities of other plant LKR/SDH enzymes [2,5,21].

### The inability of AtLKR/SDHp and AtLKRp to promote lysine synthesis within yeast cells is not due to improper intracellular localization

Although the yeast LKR has been reported to be localized in the cytosol [1], its anabolic activity appears to be relatively inefficient at the neutral pH of this compartment (Figure 5). Notably, amino acid sequence analysis revealed that this protein contains an SRL signal at its C-terminus, which functions as a peroxisome PTS1 targeting signal [22]. No such signal was present at the C-termini of either native AtLKR/SDHp or its deletion mutant encoding the monofunctional AtLKR. Thus, although AtLKRp lacks anabolic activity *in vitro*, we wished to rule out the possibility that the failure of AtLKR/SDHp and AtLKRp to complement the yeast lys1 mutant was due to improper intracellular localization within the yeast cells. To address this issue, three additional constructs were made. The first encodes a deletion mutant of yeast LKR lacking the SRL sequence, and the other two encode AtLKR/SDHp and AtLKRp to which the SRL sequence was added at their C-termini. These three constructs successfully expressed LKR polypeptides in yeast cells (results not shown), but, as shown in Figure 6, the yeast LKR lacking the SRL sequence still complemented the lys1 mutant, whereas both *Arabidopsis* LKR forms containing this sequence did not. To further confirm the cytosolic localization of the yeast and *Arabidopsis* LKR enzymes, yeast lys1 cells expressing the

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yeast or *Arabidopsis* monofunctional LKR enzymes were homogenized in a buffer containing sorbitol and their post-nuclear supernatants were fractionated on a 15–50% (w/v) sucrose gradient. Both proteins banded at the top two to three fractions of the gradient, as expected for cytosolic proteins (Figure 7).

## DISCUSSION

### AtLKR/SDHp possesses both anabolic and catabolic SDH activities, but only catabolic LKR activity

The $\alpha$-aminoadipic acid pathway is of a particular interest from a regulatory stand point, since it functions in opposite directions in different organisms – anabolically in yeast and fungi and catabolically in animals and plants. In yeast, the anabolic flux via SDH and LKR is puzzling because, *in vitro*, both enzymes can operate bi-directionally [18,23,24]. Moreover, the anabolic activity of the yeast ScLKRp is very similar to the catabolic activity of ScSDHp, in terms of both substrate utilization (saccharopine and $NAD^+$) and pH optima. Thus, in yeast cells, saccharopine, once produced by the anabolic activity of SDH, may be competitively utilized either by the anabolic activity of LKR (forming lysine and 2-oxoglutarate) or by the catabolic activity of SDH (forming $\alpha$-aminoadipic semialdehyde) [18,23,24]. In yeast, the fate of saccharopine is likely to be determined by the complex transcriptional and post-transcriptional control of genes encoding various enzymes of the $\alpha$-aminoadipic acid pathway. This apparently results in particular concentrations of intermediate metabolites that allow anabolic flux in this pathway [10,11].

The mechanisms controlling the catabolic flux of the $\alpha$-aminoadipic acid pathway in plants are still not known. However, the bi-directional operation of LKR and SDH in plants would be expected to cause the same kind of competition between the catabolic SDH and anabolic LKR activities for their common substrate saccharopine, as found for the yeast enzymes. This issue is difficult to approach biochemically, since the plant LKR and SDH enzymes are linked on a single bifunctional polypeptide and, therefore, their potential competing activities cannot be resolved. To overcome this inherent biochemical constraint, we tested whether a plant bifunctional LKR/SDH enzyme (AtLKR/SDHp) could complement defects in the anabolic SDH and LKR reactions in yeast. Our results showed that while AtLKR/SDHp complemented the yeast SDH defect, it did not complement the LKR defect. This implies that, whereas the SDH domain of AtLKR/SDHp can operate bi-directionally, the LKR domain of this bifunctional enzyme does not possess any anabolic activity. Expression of AtLKR/SDHp in yeast cells also caused significant overproduction of lysine (results not shown), supporting the notion that the *Arabidopsis* SDH can function anabolically.

### The lack of anabolic LKR activity is determined by the structure of the LKR domain of AtLKR/SDHp, rather than its linkage to SDH

The lack of anabolic LKR activity mat be determined by the structure of the LKR domain of AtLKR/SDHp, or by its linkage to SDH. To resolve this issue, we expressed in yeast a deletion mutant of AtLKR/SDH encoding only the LKR domain. Expression of this deletion mutant also enabled us to test directly the anabolic activity of the LKR domain, because this form did not possess the competing catabolic activity of SDH. Our results showed that the monofunctional AtLKRp could not complement the yeast LKR null mutant. Moreover, AtLKRp produced in yeast cells lacked any anabolic activity, but exhibited efficient catabolic activity, indicating that the lack of anabolic activity was not due to improper folding. Taken together, our results imply that (i) AtLKR/SDHp possesses only a uni-directional catabolic LKR activity; and (ii) this uni-directionality stems from the structural conformation of the LKR domain rather than from its linkage to SDH.

### The inability of AtLKR to complement the yeast LKR null mutant is not due to improper intracellular localization

The yeast LKR has been suggested to reside in the cytosol [1]. Hence one of the intriguing properties of this bi-directional enzyme is its basic pH optimum of anabolic activity (Figure 5), which is significantly above the physiological pH of the cytosol. This implies that the yeast LKR may be unfit to carry out efficient 'lysine-forming' activity in the yeast cytosol. For this reason, we were intrigued by the fact that the yeast LKR possesses a C-terminal SRL sequence, which has been proven empirically to serve as a peroxisomal targeting signal [22]. Our results showed that, despite the presence of this signal, the yeast LKR was localized in the cytosol, suggesting that this signal was not sufficiently exposed to be detected by peroxisome targeting machinery. Moreover, our results also showed that addition of a C-terminal SRL sequence to AtLKRp did not overcome the inability of this construct to complement the yeast LKR null mutant, ruling out the possibility that this inability was due to improper subcellular compartmentation.

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