Equilibrium Dissociation and Unfolding of Nucleoside Diphosphate Kinase from Dictyostelium discoideum

ROLE OF PROLINE 100 IN THE STABILITY OF THE HEXAMERIC ENZYME*

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The Killer-of-prune (K-pn) mutation in Drosophila corresponds to a Pro-Ser substitution in nucleoside diphosphate kinase (Lascu, I. Chaffotte, A., Limbourg-Bouchon, B., and Véron, M. (1992) J. Biol. Chem. 267, 12775–12781). We investigated the role of the equivalent proline (Pro100) in the formation and stability of the Dictyostelium nucleoside diphosphate kinase hexamers. Mutations to serine or glycine had only little effect on the properties of the native enzyme. However, the mutant drastically affected the subunit interaction in the hexamer and the ability of the isolated subunits to associate in vitro. While the wild-type hexamer inactivated and unfolded concomitantly at 5–6 M urea, the mutant proteins dissociated to monomers at 0.5–2 M urea and unfolded at 2.5–4 M urea. At intermediate urea concentrations, the unique species present in solution was a folded, partially active monomer as shown by size-exclusion chromatography, UV, fluorescence, and CD spectroscopy. Proline 100 is located in a loop involved in subunits contact. Altered conformation of the loop in P100S and P100G mutants demonstrates its crucial role in subunit assembly. We propose to explain the conditional dominance of the K-pn mutation by the presence of a monomeric form of the enzyme that would have deleterious effects in vivo.

Nucleoside diphosphate kinase (NDP kinase)1 (EC 2.7.4.6) catalyzes the phosphorylation of non-adenine nucleoside diphosphates. The mechanism of the reaction is ping-pong, with the formation of a phosphorylated histidine intermediate during the catalytic cycle (Garces and Cleland, 1969; Parks and Agarwal, 1973) according the following reactions:

\[
E + NTP \rightleftharpoons E\cdot P + N_{3}DP \quad \text{(Reaction 1a)}
\]
\[
E\cdot P + N_{2}DP \rightleftharpoons E + N_{3}TP \quad \text{(Reaction 1b)}
\]
\[
N_{t}TP + N_{2}DP \rightleftharpoons N_{3}DP + N_{3}TP \quad \text{(Reaction 1c)}
\]

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1 The abbreviations used are: NDP kinase, nucleoside diphosphate kinase; P100S and P100G, Pro100 mutations to Ser100 and Gly100, respectively.

2 The Dictyostelium NDP kinase is 3 residues longer than the Drosophila enzyme and 4 residues longer than the mammalian NDP kinases. According to the sequence alignment, these extra residues appear at the NH2 terminus. Therefore, the position of homologous residues varies slightly in the different NDP kinases.

3 S. Moré, I. Lascu, C. Dumas, G. LeBras, P. Brozzo, M. Véron, and J. Janin, manuscript submitted.

4 M. Chiadni and J. Janin, personal communication.

5 A. Shearn, personal communication.

The cDNA coding for a Dictyostelium NDP kinase was cloned and the enzyme overexpressed in Escherichia coli (Lacombe et al., 1990). The NDP kinases from various eukaryotic organisms are highly homologous (Wallet et al., 1991; Gilles et al., 1991). It is therefore likely that all NDP kinases fold in a similar way and important residues are conserved. This is the case for the histidine residue phosphorylated during catalysis which is His122 in Dictyostelium NDP kinase (Wallet, 1992; Dumas et al., 1992) and with15 in the human NDP kinases (Gilles et al., 1991).2 The tri-dimensional structure of a mutant NDP kinase from Dictyostelium has been solved at high resolution (Dumas et al., 1992) showing that the subunits (Mr = 16,800) are arranged in a symmetrical hexamer. The same structure was found recently in the wild-type Dictyostelium enzyme3 and a very similar structure was also found for the Drosophila NDP kinase.4

The human NDP kinases have been shown to renature efficiently after urea denaturation. Indeed, the two human homo-hexameric NDP kinases were prepared from a mixture of hetero-hexamers by ion-exchange chromatography in the presence of urea, followed by preparative renaturation (Gilles et al., 1991). Recently, we reported some properties of the wild-type NDP kinase from Drosophila and from the natural mutant Killer-of-prune (K-pn) (Biggs et al., 1988) in which proline 97 (conserved in all known NDP kinases)5 is mutated to a serine (Lascu et al., 1992).6 The stability toward heat and denaturing agents of K-pn mutant NDP kinase was dramatically decreased, and it was unable to reassemble into active hexamers after denaturation (Lascu et al., 1992). In Dictyostelium NDP kinase, this proline (Pro100) is located in a loop at the interface between subunits (that we called “K-pn loop”), and its main chain carbonyl is hydrogen-bonded to the lysine 35 from a neighboring subunit (Dumas et al., 1992). In order to obtain more information on the role of the K-pn loop in subunit association, we used site-directed mutagenesis to change proline 100 into a serine in order to produce a mutation analogous to the K-pn mutation in Drosophila. The proline to glycine mutation was also made since we reasoned that it might bring about an even more pronounced alteration of the stability than the P100S substitution (Alber, 1989).
We report here an analysis of the dissociation and unfolding properties of wild-type NDP kinase from Dicyostelium and of the mutants P100S and P100G. The study was conducted using fluorescence, UV, and CD spectroscopy, activity measurements, and size-exclusion chromatography. Preliminary experiments showed that the P100G and P100S mutants had similar properties. Therefore, in some cases only the P100G enzyme was studied in detail and compared with the wild-type protein.

**EXPERIMENTAL PROCEDURES**

**Materials**

Solutions of 10 mM urea were depleted of fluorescent impurities and salts by stirring overnight with 7 g/liter activated charcoal (Merck, Darmstadt) and 14 g/liter mixed bed resin (Dowex 50W, 20-50 mesh, Sigma), filtered through 0.22-µm Milipore filters, stored frozen, and used within 1 week. 8-Bromoinosine 5'-diphosphate was synthesized and purified as described (Lascu et al., 1979). ATP, lactate dehydrogenase, and pyruvate kinase were from Boehringer Mannheim. dTDP was from Sigma.

**Proteins**

Wild-type NDP kinase was expressed in bacteria (Lacombe et al., 1990) and purified by negative adsorption on DEAE-Sephaloc at pH 8.6 and affinity chromatography on Blue Sepharose at pH 7.4, as described (Wallet et al., 1990), except that the enzyme was eluted with 1.5 mM ATP, and MgATP was omitted from all buffers. The in vitro mutagenesis was performed essentially as described by Kunkel et al. (1987), using the oligonucleotides GCC TCA GCC GTA CTA ATT CG for the P100S and GCC TCA GCC GGA GTA CTA ATT CG for the P100G mutants (the altered nucleotides are underlined). The absence of additional mutations was ascertained by sequencing the whole coding sequence. The mutant proteins were expressed and purified using the same protocol as for the wild-type protein. The final enzyme preparations were homogeneous as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Proteins were stored frozen at -15 °C.

The enzyme concentration was estimated using an extinction coefficient of 0.6 at 260 nm in 1-cm path length for a 1 mg/ml protein solution (Wallet, 1992). The same value was used for the mutant proteins. The molar enzyme concentrations are expressed as subunit concentrations, computed from a molecular mass of 16,800.

**Methods**

**Activity Assay**—NDP kinase activity was measured by the coupled assay using 8-bromoinosine 5'-diphosphate or dTDP as acceptor and the kinetic parameters derived as previously described (Lascu et al., 1992). This assay was used for measuring the residual activity during inactivation by urea. The reaction was linear for several minutes, demonstrating no reactivation during the time of the assay. The stoichiometry of the phosphorylation was determined by a spectrophotometric method (Waygood et al., 1984; Lascu et al., 1983) based on the trapping of the ADP formed in Reaction 1a by the reactions catalyzed by pyruvate kinase and lactate dehydrogenase. Alternatively, the incorporation of radioactive phosphate from [γ-32P]ATP into the protein was measured. In this case, the phosphorylated protein was separated from excess ATP by a modification of the gel filtration-centrifugation technique (Ponenksy, 1975). For this, 100-µl solution aliquots were applied to 1-ml columns of QAE Sephadex A-25 equilibrated with 50 mM HEPES buffer, pH 6.5, containing 8 M urea, centrifuged, and washed with 0.5 ml of the same buffer allowing elution of the phosphorylated enzyme while ATP, ADP, and P, bound to the resin.

**Absorbance Measurements**—UV spectra of NDP kinases were recorded at 25 °C in 80 mM Tris-acetate, pH 8.0, with a Perkin-Elmer Lambda 5 spectrophotometer in the 240-400-nm range. The protein concentration was 0.15 mg/ml. The second derivatives of the spectra were calculated using a window of 2 nm.

**Intrinsic Fluorescence Measurements**—The emission fluorescence spectra of the enzymes (about 100 µg/ml) were recorded upon excitation at 295 nm (2.5 nm bandpass for both excitation and emission) using a Perkin-Elmer LS5B instrument. The quenching of the intrinsic protein fluorescence by acrylamide and iodide was studied as described (Calbou et al., 1983; Prasad et al., 1988). The Stern-Volmer constants (Eftink and Ghiron, 1981) were derived from the plot of F/F0 as a function of the concentration of acrylamide and NaF, respectively, after correction for the inner filter effect of acrylamide (Yagi and Simon, 1992). Corrections for this effect and for the dilution were less than 10%.

**Circular Dichroism Spectroscopy**—Circular dichroism spectra at a protein concentration of 0.2 mg/ml were recorded on a Jasco JYI CD6 instrument at 20 °C in 50 mM Tris-HCl buffer, pH 7.5, with a cuvette of 0.1-cm path length, in the 200-240-nm range.

**Size-exclusion Chromatography**—Samples (100 µl) were analyzed on a Superose 12 column equilibrated in 83 mM Tris acetate, pH 8.0, containing 0.2 M NaCl at a flow rate of 0.7 ml/min, using a fast protein liquid chromatography system of Pharmacia. The column was calibrated as described by Corbett and Roche (1984). When using the denaturant-gradient technique of Endo et al. (1983), a flow-rate of 1.0 ml/min and an urea gradient of 30 mM/ml were used over 200 min.

**Equilibrium Denaturation/Renaturation Experiments**—The NDP kinase was diluted at the final concentration of 150-200 mM/ml in 83 mM Tris acetate buffer containing the indicated concentrations of urea and incubated 2-24 h at 25 °C. The renaturation was studied in the same way, except that the protein samples were denatured during 3 h in 10 M urea before dilution as above. The fluorescence spectra indicated that the unfolding was complete within a few seconds in 10 M urea.

**Data Analysis**—The equilibrium constant of unfolding was calculated assuming a two-state model. Free energy changes were estimated according to Equation 1 (Schjerst et al., 1992; Jackson and Fersht, 1991).

\[
\Delta G_{\text{fold}} = \frac{F_u - F_x}{1 + \exp(m[\text{urea}])} = \frac{F_u - F_x}{1 + \exp(m[\text{urea}])} \Delta G_{\text{fold}}/RT.
\]

**RESULTS**

**Enzymatic and Spectroscopic Properties of the Native Wild-type and Mutant NDP Kinases**—Recombinant P100S and P100G mutant NDP kinases were purified and their kinetic parameters were measured. As shown in Table I, values for \(k_{\text{cat}}\) and \(K_m\) were similar for the wild-type and mutant enzymes. When using the ATP regenerating system, the stoichiometry of phosphorylation was close to one phosphate/subunit with all enzyme preparations. The lower phosphorylation ratios found in the past by other authors can be explained by the fact that the equilibrium constant of Reaction 1a is approximately 0.2 for the yeast and pig NDP kinases (Garces and Cleland, 1969; Lascu et al., 1983). It is therefore necessary to use an ATP regeneration system to shift the reaction toward completion.

**TABLE I**

<table>
<thead>
<tr>
<th>Kinetic parameters of wild-type, P100S, and P100G mutant nucleoside diphosphate kinases from Dicyostelium</th>
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<tr>
<td><strong>Kinetic constant</strong></td>
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<td><strong>K_m (s^-1)</strong></td>
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*Based on a molecular mass of 16,800.
The P100G mutant NDP kinase displays UV and fluorescence spectra similar to the wild-type enzyme (not shown). The dichroic spectra of the two proteins are also similar and are characterized by a broad negative peak at 218 nm which is typical of an (α/β) protein (see below) (Hennessy and Johnson, 1981). The Stokes radii of the wild-type and mutant NDP kinases were found to be identical by gel filtration on a calibrated Superose 12 column (data not shown). Previous investigations using both gel filtration and ultracentrifugation showed that Dictyostelium NDP kinase is a hexamer in solution (Wallet, 1992), as well as in crystal state (Dumas et al., 1992). We conclude that native P100S and P100G mutant enzymes are hexamers and that their conformation and activity are very similar to those of the wild-type enzyme.

Transitions Monitored by Enzymatic Activity and Protein Phosphorylation—Upon incubation for 24 h in various concentrations of urea, wild-type NDP kinase was inactivated between 5 and 6 M urea (Fig. 1). The reactivation is completely reversible with a midpoint transition at 3 M urea. Mutation of proline 100 had a dramatic effect on the enzyme stability: the P100S mutant lost most of its activity at 1.5 M urea, while the P100G mutant was inactive at 0.7 M urea. Under our standard reactivation conditions, the yields of the reactivated mutant enzymes were lower than for the wild-type enzyme.

In a separate experiment, the P100G mutant enzyme was first dissociated with 2 M urea or fully unfolded with 6 M urea, and then urea was totally removed by gel filtration on a Sephadex G-25 column. In both cases, the recovery of activity reached about 30% of control samples in 24 h. The reactivation yield could be improved by adding 1 mg/ml bovine serum albumin and 1.5 mM ATP to the reactivation mixture. The P100G mutant enzyme then recovered about 75% of its activity in 2 h (not shown).

Residual activity after incubation in urea was independent of protein concentration from 10 to 250 μg/ml (not shown). A low but significant residual activity of about 0.5–0.7% was measured after incubation of P100G NDP kinase in the presence of intermediate urea concentrations. Two types of experiments demonstrated that the P100G mutant enzyme could be phosphorylated under these conditions, i.e. that Equation la occurs. First, incubation with [γ-32P]ATP (Fig. 1B) led to the incorporation of [32P]phosphate in the protein at urea concentrations well above the transition detected by activity. Second, protein phosphorylation was measured with the coupled system used for activity measurements lacking the diphosphate acceptor dTDP (Lascu et al., 1983). Thus, ADP produced in Reaction 1a was trapped by the pyruvate kinase reaction, and the pyruvate produced was quantified using the lactate dehydrogenase by following the decrease in NADH. The recovery of activity after urea dilution was measured in a separate experiment. As shown in Fig. 1B, the enzyme was phosphorylated within 2 min, whereas the recovery of activity during this time period was less than 7%. It should be noted that, when using this protocol, the rate of the process has little significance since it is limited by the activity of the coupled system. The slow absorbance drift reflected the nucleoside triphosphatase activity of NDP kinase (the non-enzymatic hydrolysis of the phosphohistidine intermediate), but the possibility of contamination with ATPase(s) cannot be excluded. We conclude that at 0.7–2.0 M urea P100G mutant loses 99.5% of its phosphotransferase activity but retains its ability to autophosphorylate in the presence of ATP.

It is important to note that in the experiments presented in Fig. 1 the residual reaction rate was measured under catalytic conditions (Reaction 1c), whereas in the Figs. 1B and 2 the measurement of the stoichiometry of phosphorylation-
Dissociation and Unfolding of Dictyostelium NDP Kinase

Size-exclusion Experiments—In order to examine whether inactivation of the mutant enzymes at low urea concentration was due to dissociation of the hexamers, we have investigated the quaternary structure of wild-type and P100G mutant by size-exclusion chromatography on Superose 12 at various urea concentrations. We use the term “dissociation” for the transition from the native hexamer to a folded species of lower molecular mass, whereas “denaturation” or “unfolding” qualifies the transition from the dissociated species to the unfolded polypeptide chain. The column was calibrated at various urea concentrations according to Corbett and Roche (1984). Patterns of dissociation and unfolding by urea obtained for the wild-type enzyme were different from those found for mutant. With the wild-type NDP kinase, no species with a Stokes radius lower than that of the hexameric native enzyme was detected, indicating that the dissociation was immediately followed by unfolding. The fully unfolded monomer happens to have a Stokes radius similar to that of the native hexameric enzyme (not shown).

On the contrary, P100G mutant NDP kinase dissociated at low urea concentrations. Fig. 3, left panel, shows that in the 0–0.7 M range, the native hexameric protein (elution volume 11.4 ml, marked by an asterisk) gradually disappears as the urea concentration increases, while another species with a larger elution volume (13.8 ml, marked by a circle) is formed. The assignment to the monomer of the unique species present between 0.7 and 2.0 M urea is unambiguous since among all possible intermediates (dimers, partially unfolded monomers, and folded monomers) the folded monomer has the lowest Stokes radius. Its elution volume is the same as that of myoglobin (M, 17,000) used as marker protein (not shown). No other species (soluble aggregate or protein precipitate) was observed at any urea concentration. We conclude that the intermediate species present at 0.7–2 M urea is the folded monomer. At urea concentrations higher than 2 M, the elution volume of the single protein peak changes to lower values corresponding to larger hydrodynamic volumes. This indicates that P100G is undertaking an equilibrium unfolding transition, the single peak reflecting a fast equilibrium between unfolded and folded monomers. In contrast, dissociation cannot be described as an equilibrium process at the time scale and protein concentrations used in this study, since two protein peaks are present. When analyzing the molecular species present during protein refolding, a similar pattern was observed, except for a lower amount of hexameric protein (Fig. 3, right panel).

The same behavior was observed when the dissociation of the P100G enzyme was analyzed by denaturant-gradient size-exclusion chromatography according to Endo et al. (1983) (not shown). Here the protein exposure to denaturant was only 12–14 min as compared to 24 h in the experiment described in Fig. 3. Between 2–6 M urea, only one protein peak was again observed, and the variation of its elution volume corresponds to unfolding of the protein.

These data show that at intermediate urea concentrations ranging from 0.7 M to 2.0 M, the P100G NDP kinase is monomeric and retains a compact structure.

Spectroscopic Studies—The UV spectra of P100G mutant enzyme in the absence of urea and in the presence of 2 and 6 M urea are shown in Fig. 4. The UV absorption spectrum of the monomeric intermediate which accumulates in 2 M urea upon either denaturation or renaturation is very similar to that of the native enzyme. However, the second derivative spectra clearly indicate a difference in the peaks at 276 ± 0.5, 281 ± 0.5, and 285 ± 0.5 nm. These changes are probably due to changes in the environment of Tyr'54 which is located in the contact area between the subunits (Dumas et al., 1992). It should be noted that the shoulder at 305 nm present in the native and monomeric NDP kinases is absent in the denatured enzyme.

Dictyostelium NDP kinase has a single tryptophane residue which makes it suitable to monitor changes in the protein conformation. Upon denaturation, the intrinsic fluorescence was considerably quenched, and the maximum of the spectrum was shifted from 335 to 350–355 nm (Fig. 5). In the presence of 0.7–2.0 M urea, the mutant proteins had fluorescence spectra very similar to those of the native proteins,

![Fig. 3. Size-exclusion chromatography analysis of denaturation/renaturation by urea.](image)

![Fig. 4. UV spectra (A), P100G mutant NDP kinase, in 0 M (solid line), 2 M (dashed line), and 6 M urea (dotted line) and (B) second derivative spectra, with the same symbols. The protein concentration was 150 μg/ml. Peaks marked with asterisks have a significantly different amplitude in the absence or in the presence of 2 M urea.](image)
Perform a quantitative treatment of the data. Assuming a was considerably more exposed in the denatured state. The same Stern-Volmer constant in mutant and wild-type proteins (1.97 M\(^{-1}\)). The intrinsic protein fluorescence was quenched by acrylamide and/or flexibility of the polypeptide chain in the native protein. Pro'm does not elicit important changes of conformation.
Dissociation and Unfolding of Dictyostelium NDP Kinase

Effect of the Substitution of Proline 100 on the Stability and Conformation of NDP Kinase—The wild-type hexameric enzyme inactivated (probably reflecting dissociation) and unfolded (as shown by fluorescence and CD transitions) concomitantly between 5 and 6 M urea. However, the refolding of the protein upon dilution of denaturant occurred only at 2-3 M urea, showing the hysteretic behavior frequently observed with oligomeric proteins (Jaenicke, 1987). It may be due to the presence of assembly intermediate(s) in which the polypeptide chain is less stable to denaturation as compared to the polypeptide within the oligomeric protein (Fuchs et al., 1991).

Mutation of proline 100 in Dictyostelium NDP kinase into serine or glycine results in altered stability of the hexameric enzyme. In the case of the P100S and P100G mutated enzymes, inactivation transition proceeded at lower urea concentrations than unfolding, as shown by fluorescence and ellipticity changes, indicating the occurrence of an intermediate. Substitutions in small monomeric proteins of prolines by amino acids with smaller side chains, and/or of any amino acid by glycine, are expected to have destabilizing effects by stabilization of their unfolded state (Alber, 1989). However, in some cases the mutation of prolines does not affect the activity of the native protein but rather has local effects; the
loss of interactions within the protein region containing the proline leads to an alteration of the function at denaturant concentrations where the over-all tri-dimensional structure of the protein is mostly conserved (Koshy et al., 1990; Schjelderup et al., 1992). For example, in the case of the P30A mutants in cytochromes c from several species the protein conformations as probed by the absorption band at 695 nm were susceptible to concentrations of urea that had little influence on their overall structure (Koshy et al., 1990). Our results clearly indicate a similar consequence of the mutation of proline 100 into serine or glycine in Dictyostelium NDP kinase. Indeed, wild-type and mutant native NDP kinases have similar specific activities, quaternary structure, and conformation, as probed by several spectroscopic signals. Moreover, the quenching of the intrinsic protein fluorescence was described by identical $K_{sv}$ values for the wild-type and P100G mutant, indicating a similar flexibility of their polypeptide chain in the native state.

**Properties of the Monomeric Intermediate of the Mutant NDP Kinases**—The results presented above indicate that at low urea concentrations (0.7–2 M) the mutant NDP kinase exist as a folded compact monomer. Several lines of evidence show that the conformation of this monomeric intermediate was similar to that of the native protein, since: (i) it could be fully phosphorylated and had residual activity; (ii) its UV absorption, fluorescence, and CD spectra were typical for a folded protein and were very similar to those of the native protein and; (iii) the single tryptophane residue present in the sequence had an accessibility to quenchers similar to that of the native protein.

We propose to explain the dissociation properties of the P100S and P100G mutant NDP kinases by an increased flexibility of the K-pn loop. In the wild-type protein, the conformation of this large loop could be locked, even in the monomeric state, in a conformation similar to that present in the hexamer, allowing a fast subunit assembly. Mutation of the proline 100 to glycine or to serine could induce flexibility of the loop thus decreasing the occupancy of the conformation competent for assembly. It is to be expected that this effect would be amplified by the multistep nature of the assembly process. Despite these differences, the properties of the monomeric enzyme show that its overall conformation is close to native. Furthermore, the P100S and P100G have identical free energies of unfolding, indicating again that Pro$^{100}$ is not involved in the “core” structure of the subunits.

We believe that our results are important in the context of the existence of the natural Drosophila mutant K-pn (Sturtevant, 1955) which leads to a P97S replacement in the Drosophila NDP kinase (Lascu et al., 1992). By analogy with the properties of the Dictyostelium P100G mutant described in this study, we propose that the K-pn mutation might lead to the presence of monomers in the Drosophila cells. The validity of this analogy is supported by our previous study showing that the P97S NDP kinase from K-pn flies is fully active but less stable than the wild-type enzyme (Lascu et al., 1992) and by the high level of sequence homologies within the K-pn loop between Dictyostelium and Drosophila NDP kinases (Dumas et al., 1992). Moreover, preliminary results indicate that the structure of the Drosophila and Dictyostelium NDP kinases are nearly identical.

The most intriguing aspect of the K-pn mutation is its dominant lethal interaction with the prune gene, a property not easily explained at the biochemical level. Flies homozygous for K-pn have a wild-type phenotype. In contrast, larvae die even when heterozygous for K-pn when the prune gene is mutated although the pn mutation is itself not lethal (Biggs et al., 1988; Hackstein, 1992). prune has recently been cloned (Teng et al., 1991) but its function is not clearly established (Barnes and Birglin, 1992). Interestingly, prune larvae heterozygous for the K-pn mutation contain about 40% wild-type NDP kinase activity as characterized by its thermostability. Thus, the lethal phenotype is not due to the lack of wild-type NDP kinase (see also Lifschytz, E. and R. Falk, 1969). We propose that the “neomorphic” function of the mutant K-pn protein results from deleterious effects of monomeric NDP kinase present in K-pn larvae. The monomeric form of the enzyme could interact with other cellular components through protein regions less exposed in the hexameric enzyme. In addition, translocation of the monomeric species to the wrong cellular compartment or its secretion outside the cell could occur. An extracellular factor which inhibits the differentiation of lymphocytes has been identified as a NDP kinase (Okabe-Kado et al., 1992), and, recently, the existence of NDP kinases on the cell surface has been demonstrated (Urano et al., 1989). An example of reduced stability to denaturation that is associated with increased efficiency of excretion is provided by a mutant of human lysozyme expressed in yeast (Taniyama et al., 1992). Using gynandromorphs of Drosophila to do cell fate mapping, Orevi and Falk (1975) noted that the focus of the pn/K-pn interaction is domineering, that is, that.

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*I. Lascu, unpublished results.*
patches of cells genotypically lethal could induce lethality to neighboring cells which were genotypically non-lethal. Also relevant is the recent demonstration of a tumor suppressor function of auxin on the hematopoietic Drosophila oncogene Turn-1 (Zinyk et al., 1993). Although an attracting hypothesis, the ability of K-1n NDP kinase monomers to cross biological membranes remains to be established.

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