Role of AWD/Nucleoside Diphosphate Kinase in *Drosophila* Development

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The *abnormal wing discs* gene of *Drosophila* encodes a soluble protein with nucleoside diphosphate kinase activity. This enzymic activity is necessary for the biological function of the *abnormal wing discs* gene product. Complete loss of function, i.e., null, mutations cause lethality after the larval stage. Most larval organs in such null mutant larvae appear to be normal, but the imaginal discs are small and incapable of normal differentiation. *Killer-of-prune* is a neomorphic mutation in the *abnormal wing discs gene*. It causes dominant lethality in larvae that lack *prune* gene activity. The *Killer-of-prune* mutant protein may have altered substrate specificity. Null mutant larvae have a low level of nucleoside diphosphate kinase activity. This suggests that there may be additional *Drosophila* genes that encode proteins with nucleoside dipthosphate kinase activity. Candidate genes have been found in the *Drosophila* genome.

THE *abnormal wing discs* GENE OF *Drosophila* ENCODES A NUCLEOSIDE DIPHOSPHATE KINASE

During embryogenesis of holometabolous insects such as *Drosophila melanogaster*, the somatic cells are segregated into two lineages; larval cells and imaginal cells. The larval cells stop dividing during embryogenesis and differentiate into larval organs. During larval life these larval cells grow by increasing in cell size; their nuclei become polytene. By contrast, the imaginal cells during larval life remain diploid and continue to divide by normal cell division. During metamorphosis, which occurs in the pupal stage, most of the larval cells are histolyzed or are removed by other processes, while the imaginal tissues differentiate into adult organs. The dramatic result is that the adult animal, which emerges from the pupal case, is completely different in morphology, physiology, and behavior than the larval animal.

In order to identify genes specifically required for imaginal disc development, several labs have screened for lethal mutations that cause death at the end of the larval stage or during the pupal stage (Shearn *et al.*, 1971, 1972; Kiss *et al.*, 1976). The *abnormal wing discs* (*awd*) gene was identified in just such a screen (Dearolf, 1986). The *awd* gene is located in the distal region of the right arm of the third chromosome within the 100 D3 region. The *awd*^KRs6^ mutation is a null or amorphic allele; it is missing the entire coding region (Timmons *et al.*, 1995). All of the imaginal discs from *awd*^KRs6^ homozygous larvae are extremely small and have no capacity to differentiate when transplanted into metamorphosing larval hosts (Hersperger and Shearn, unpublished observation). The failure of *awd* mutant imaginal discs to differentiate when transplanted indicates that the requirement for *awd* gene expression is disc-autonomous. Somatic recombination studies demonstrated that the requirement for *awd* gene expression in wing, eye-antenna, and leg imaginal

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discs is also cell-autonomous (Dearolf *et al.*, 1988a). The *awd* gene is not only required for imaginal disc development, it is also required in the imaginal cells of the brain and lymph glands and for ovarian development (Dearolf *et al.*, 1988a).

The *awd* mutant gene was cloned from a genomic library made from heterozygotes of the original dysgenic allele (Dearolf *et al.*, 1988b). The mutant gene was then used to screen a genomic library for the wild-type gene. The *awd* gene encodes a single 0.8-kb transcript, which is present throughout development. In wild-type late third instar larvae, the transcript accumulates in all of the tissues, which are defective in mutant larvae, such as imaginal discs, brains, lymph glands, and ovaries (Dearolf *et al.*, 1988b). However, it also accumulates in larval tissues, such as the fat body, which do not appear to be defective in mutant larvae (Dearolf *et al.*, 1988b). Antibodies raised against an AWD fusion protein recognize a 17 kDa polypeptide on immunoblots that is a subunit of a 100 kDa protein (Biggs *et al.*, 1988). The nucleotide sequence of the *awd* cDNA predicts a protein that is 78% identical to the human NM23 (now known as NM23-H1) protein (Rosengard *et al.*, 1989). Antibodies raised against a peptide of NM23 cross react with *Drosophila* AWD.

Chromosomes prepared from *awd* mutant larval brains revealed an accumulation of condensed metaphase figures that are similar to chromosomes from nonmutant larval brains treated with colcemid (Biggs *et al.*, 1990). Since colcemid blocks polymerization of microtubules, it was hypothesized that the block in cell division is caused by a failure in microtubule polymerization. This led to a search of the literature for a small (~17 kDa) protein that was associated with microtubules. In fact, Nickerson and Wells (1984) had reported the isolation of nucleoside diphosphate kinase from purified bovine brain microtubules and showed that on SDS-PAGE it had an apparent molecular weight of ~17 kDa. Antibodies raised against bovine brain NDP kinase cross react with the AWD protein. Moreover, extracts of *awd* mutant larvae have much less NDP kinase activity than extracts of nonmutant larvae. These data indicated that AWD represents a *Drosophila* NDP kinase (Biggs *et al.*, 1990). Antibodies raised against a peptide of NM23 decorate *Drosophila* microtubules (Biggs *et al.*, 1990) suggesting that the mitotic defect in *awd* mutant larvae was a consequence of defective microtubule polymerization. At about the same time, the amino acid sequence of a *Dictyostelium discoideum* NDP kinase was published (Lacombe *et*

al., 1990). This was the first NDP kinase sequence to be published. The sequence of this *Dictyostelium discoideum* NDP kinase is 60% identical to *Drosophila melanogaster* AWD (Wallet *et al.*, 1990). This evidence strengthened the argument that AWD represented an NDP kinase. The best evidence is that purified recombinant AWD protein has NDP kinase activity (Timmons *et al.*, 1995).

THE NDP KINASE ACTIVITY OF AWD IS NECESSARY BUT NOT SUFFICIENT FOR BIOLOGICAL ACTIVITY

Given that the AWD protein has NDP kinase activity, one must ask whether that activity is necessary for the biological activity of the protein. This question was addressed by *in vitro* mutagenesis. Many lines of evidence suggested that the histidine at position 119 was the active site for transfer of a high-energy phosphate bond. This histidine was mutated to alanine (H119A) in a transgene that has an *awd* cDNA driven by an *awd* promoter and was tested by germline transformation for protein accumulation, NDP kinase activity, and rescue of the *awd* mutant phenotype. It was found that this mutant protein accumulated at expected levels, but it had no NDP kinase activity and failed to rescue the *awd* mutant phenotype (Xu *et al.*, 1996). This indicated that the NDP kinase activity of AWD is necessary for its biological activity. This is a significant result because there are some reports that either NM23-H1 or NM23-H2 with the active site histidine mutated retains some biological activity (MacDonald *et al.*, 1993; Postel and Ferrone, 1994).

Given that the NDP kinase activity of AWD is necessary for its biological activity, one must ask whether that activity is sufficient for the biological activity of the AWD protein. This question was addressed by examining the ability of human NM23/NDP kinases to rescue the *awd* null mutant phenotype. Transgenes were constructed in which the only human sequences were the protein coding regions of either *nm23-H1* or *nm23-H2*. These transgenes had the 750-bp *awd* promoter and 5′ and 3′ transcribed but untranslated sequences from an *awd* cDNA. Since both human NM23/NDP kinases differ from AWD/NDP kinase by 22% of the amino acids, these transformation experiments with heterologous cDNAs represent a crude kind of mutagenesis. The control for these heterologous transgenes, the homologous transgene, rescues every aspect of the mutant phenotype. The 750-bp *awd* pro-

moter fused to *lac*Z had previously been used to examine the normal pattern of *awd* expression (Timmons *et al.*, 1993). The temporal and tissue specific pattern of expression of this reporter gene as monitored by β-galactosidase accumulation in a wild-type background was similar to expression of the *awd* transgene as monitored by immunohistochemistry or by RNA *in situ* hybridization in an *awd* null mutant background. Rescue of the *awd* null phenotype by the *awd* transgene indicated that all of the required regulatory sequences were included in the construct and that substituting the human coding regions would produce meaningful results. Three transformants with *nm23-H1* and two with *nm23-H2* were recovered and assayed both for NDP kinase activity and for rescue of the mutant phenotype. All of the transformants accumulated NDP kinase activity indicating that both of these human cDNAs are expressed in flies and their products are enzymically active. While both human transgenes rescued the mutant phenotype to some extent, the *nm23-H1* transgene did not rescue the zygotic lethal phenotype as completely as did the *nm23-H2* or *awd* transgene and neither the *nm23-H1* nor the *nm23-H2* transgene rescued the female sterile phenotype as completely as did the *awd* transgene (Xu *et al.*, 1996). These results support the hypothesis that the biological activity of AWD depends on its NDP kinase activity and its interaction with other cellular proteins which, for example, could require nucleoside triphosphates for their function. According to this hypothesis, the human proteins expressed in flies have adequate NDP kinase activity but are defective in interacting with fly cellular proteins. If the biological activity of AWD depended solely on its nucleoside diphosphate kinase activity, then heterologous transgenes, which expressed the same level of NDP kinase activity as the homologous transgenes, should rescue the mutant phenotype as well as the homologous transgene.

There are many reports in the literature suggesting that NDP kinases provide local supplies of GTP for proteins that require GTP (e.g., Kimura and Johnson, 1983; Wieland and Jakobs, 1992). The evidence of an association of NDP kinase with microtubules suggests that this NDP kinase may be providing a localized supply of GTP for microtubule polymerization. Recent genetic evidence may support such a role. The *shibire* gene encodes the *Drosophila* homolog of dynamin, a GTP-binding protein involved in endocytosis (Chen *et al.*, 1992). Null mutations are lethal, so *shibire* is a vital gene. However, some temperature-sensitive mutations of *shibire* cause paralysis when mutant adults are shifted to a nonpermissive temperature. In a screen for enhancers of *shibire* mutations, *E(shi)*, ones that lower the temperature at which temperature-sensitive mutations cause paralysis, mutations in the *awd* gene were identified. NDP kinase activity is reduced in these *E(shi)A* mutants and the enhancer phenotype is rescued by an *awd* transgene (Ramaswami, personal communication). This exciting new evidence may be an important clue as to the biological function of AWD. It suggests that AWD may provide a local supply of GTP for SHIBIRE/DYNAMIN function, although, it does not rule out that AWD could be providing a global supply of GTP.

DOES AWD HAVE ACTIVITIES OTHER THAN NDP KINASE?

It has been reported that AWD/NDP kinase also has protein kinase activity (Inoue *et al.*, 1996). Supernatants of wild-type larval extracts incubated with [^{35}S]GTPγS lead to the thiophosphorylation of several proteins. However, supernatants of *awd* mutant larval extracts incubated with [^{35}S]GTPγS does not lead to the thiophosphorylation of any proteins. This was interpreted as evidence that AWD/NDP kinase has protein kinase activity. However, another interpretation of this data is that AWD/NDP kinase transfers the labeled moiety of [^{35}S]GTPγS to ADP and proteins in the supernatant are thiophosphorylated by conventional protein kinases and [^{35}S]ATPγS. According to this interpretation, no proteins in the mutant supernatant are labeled, because no [^{35}S]ATPγS is generated.

POSSIBLE ROLE OF AWD/NDP KINASE IN *Drosophila* TUMORS

There are several recessive lethal mutations in *Drosophila* that lead to the overgrowth of neural and/or imaginal disc and/or blood cells (Gateff and Mechler, 1989). The overgrowth phenotype caused by such mutations has been described as tumorous or neoplastic in those cases where the overgrowth is accompanied by loss of normal tissue structure and loss of capacity for normal differentiation. Since the absence or reduced level of function of the genes identified by these mutations leads to the formation of neoplastic tumors, the genes have been called tumor suppressor genes (Gateff and Mechler, 1989). Because of the evidence of a role for the *nm23* gene in mammalian

tumors, the possible role of the *awd* gene product in *Drosophila* tumors caused by mutations in such tumor suppressor genes has been investigated.

EFFECT ON *awd* GENE EXPRESSION OF MUTATIONS IN TUMOR SUPPRESSOR GENES

Mutations in *lethal giant larvae* cause brain tumors. *awd* expression in tumorous brains from *lethal giant larvae* mutant hemizygotes was assayed by measuring NDP kinase activity in extracts, by immunoblotting and by examining β-galactosidase accumulation from an *awd-lacZ* reporter transgene. The *awd* gene is overexpressed in tumorous brains from *lethal giant larvae* (Timmons *et al.*, 1993). Compared to normal brains, the specific activity is moderately increased in tumorous brains dissected from mutant larvae (2.5×) and substantially increased in tumors that had been cultured in adult hosts (9×). Immunoblotting revealed similar increases. The immunoblotting data was quantitated in two different ways, which yielded similar results. One way was to directly capture the light emitted by chemiluminescence with a video camera (Stratagene Eagle Eye system); the other way was to capture the light on film and to do the quantitation by densitometry. In either case, to control for variation in sample size the values were normalized to the signal for α-tubulin detected on the same filter with a monoclonal antibody. The observed increase in the accumulation of AWD protein in these tumors was mirrored by increased accumulation of β-galactosidase from the *awd-lacZ* reporter transgene.

By itself the significance of the increased expression of the *awd* gene in tumorous brains from *lethal giant larvae* was not clear. It could have been that *awd* gene expression is characteristic of dividing cells and that the only dividing cells in the mutant brains were the tumor cells. In that case, expression of *awd* would only be a marker of dividing cells. The analysis of double mutants clarified the significance.

EFFECTS OF *awd* GENE MUTATIONS ON PHENOTYPE OF MUTATIONS IN TUMOR SUPPRESSOR GENES

In order to investigate the requirement for *awd* gene expression in *Drosophila* tumors, double mutant combinations were made of a severe hypomorphic *awd*

mutation (awd^{b3}) or of a null *awd* mutation (awd^{KRS6}) with null mutations in *lethal giant larvae* and in two other tumor suppressor genes, *discs large* and *brain tumor*. To recognize homozygous double mutant larvae from their heterozygous sibs, all of the stocks carry a *yellow* mutation on their X chromosomes and carry the wild-type allele of *yellow* on their balancer chromosomes. Second and third chromosome balancers carrying the wild type allele of *yellow* were generated by transposition onto existing balancer chromosomes of a transgene that expresses the wild type allele of *yellow* (Timmons *et al.*, 1993). It is crucial that the marker on these balancer chromosomes, the wild type allele of *yellow*, is stably associated with it, so double mutant homozygous larvae could be identified as those expressing the *yellow* larval phenotype. No tumors form in *lethal giant larvae; awdr*^{b3} double-mutant homozygous larvae (Timmons *et al.*, 1993). This means that some expression of *awd* is required for tumor growth. However, tumors do form in *discs large; awd*^{b3} double-mutant larvae. This indicates that the brain tumors caused by *discs large* do not require very much *awd* gene expression and it demonstrates at least a quantitative difference between these tumors and those caused by *lethal giant larvae*, which do require more *awd* gene expression than can be supplied by awd^{b3}. By contrast, all three double-mutant combinations of the *awd null* mutation, awd^{KRS6}, with *null* alleles in *lethal giant larvae*, *discs large*, or *brain tumor* cause lethality at the end of the embryonic period (Hersperger, unpublished). This is an extremely significant result. Each of the mutations by themselves causes lethality at the end of the larval period. The earlier lethality of the double mutants means that larvae missing zygotic expression of *lethal giant larvae, discs large*, or *brain tumor* can survive to the end of the third larval instar only if they have zygotic expression of *awd*. This is evidence of a functional relationship between *awd* and these tumor suppressor genes.

prune/Killer-of-prune LETHAL INTERACTION

awd gene-mutations had been located genetically near the conditional dominant mutation, *Killer-of-prune (Kpn)* (Sturtevant, 1956). In a wild-type background *Kpn* homozygotes or heterozygotes express no phenotype, however in a *prune (pn) null* mutant background, *Kpn* is a dominant lethal. Deletions of *awd* were generated by screening for gamma ray-induced

revertants of *Kpn* using a previously published approach (Lifschytz and Falk, 1969). Analysis of the *Kpn* revertants led to the thoroughly unexpected finding that *Kpn* is actually a mutation in the *awd* gene (Biggs *et al.*, 1988). This was confirmed by transforming flies with a transgene containing the *awd* gene cloned from *Kpn* homozygous flies and showing that it causes lethality in a *pn* mutant background (Biggs *et al.*, 1988). It was subsequently shown by DNA sequencing that this mutant allele, now renamed *awd^{Kpn}*, has a change of a single nucleotide, which leads to the replacement of proline by serine at residue 97 of the AWD polypeptide (Timmons *et al.*, 1995). A comparison of the amino acid composition of purified wild-type AWD protein to that of purified KPN protein confirms that this proline to serine change is the only difference between the wild-type and mutant protein (Lascu *et al.*, 1992).

Drosophila prune GENE PRODUCT

The *prune* gene was cloned several years ago (Teng *et al.*, 1991; Frolov *et al.*, 1994). The sequence of the entire PN protein predicted from a cDNA nucleotide sequence does not provide strong evidence of a biochemical function. It had previously been speculated by Teng *et al.* that PN was a GTPase-activating protein or GAP protein (Teng *et al.*, 1991). However, that speculation has not been substantiated by experimental data and an authentic GAP protein has been identified in *Drosophila* (Gaul *et al.*, 1992). Sequence analysis has suggested that PN is a member of a family called DHH phosphoesterases (Aravind and Koonin, 1998). This family includes proteins with polyphosphatase activity and ones with phosphodiesterase activity. Although genetic evidence supports the idea that PN is an enzyme (Timmons and Shearn, 1996), no biochemical evidence of a PN enzymatic activity has been reported.

It has been hypothesized that the biological activity of AWD/NDP kinase involves an association with proteins that require nucleoside triphosphates for their function (Xu *et al.*, 1996). The product of the *prune* gene was investigated as an initial candidate for an associated protein because of the dominant lethality of *awd^{Kpn}* individuals in the absence of *prune* gene function. The intracellular localization of AWD/NDP kinase was examined by immunohistochemistry in *pn* mutants. It was found that the pattern of localization that had been determined in wild-type larvae (Timmons

et al., 1993; Xu *et al.*, 1996) was unchanged in *pn* mutant larvae.

The human homolog of *prune* has recently been described (Reymond *et al.*, 1999). It was reported that this protein physically associates with human NM23-H1 and with human NM23-H1 carrying the "Kpn mutation," P96S (Reymond *et al.*, 1999). By contrast, no such physical association has been detected between *Drosophila* PN and AWD or KPN (L. Timmons, unpublished).

Tumorous-lethal (Tum-l) is a gain of function mutation in the *Drosophila hopscotch* gene (Luo *et al.*, 1997) that leads to premature differentiation and aggregation of blood cells. The dominant *awd^{Kpn}* mutation has been reported to suppress the hematopoietic defects associated with *Tum-l* (Zinyk *et al.*, 1993) and cause increased survival of *Tum-l* hemizygotes. Mutations in the *prune* gene have also been reported to cause increased survival of *Tum-l* hemizygotes. These data have been interpreted as indicating the existence of a hematopoietic regulatory pathway involving the HOP, KPN, and PN proteins.

In order to detect wild-type and mutant forms of AWD/NDP kinase on immunoblots, rabbit polyclonal antiserum was generated by immunizing a rabbit with purified, recombinant AWD/NDP kinase. High-affinity antibodies were recovered from this antiserum by affinity chromatography and are referred to as HA1. The specificity of HA1 immunoreactivity on immunoblots was tested on extracts of wild-type and *awd^{KRS6}* null mutant larvae fractionated by SDS-PAGE or by agarose isoelectric focusing. HA1 recognizes a single band at 17 kDa in extracts of wild-type larvae that is absent in *null* mutant larvae on immunoblots of SDS-PAGE gels; no other protein bands are visible. This antibody is, therefore, very specific for the AWD/NDP kinase protein. The HA1 antibody recognizes a major band at a pI of 7.4 and minor bands at pI's of 7.7 and 7.1 in extracts of wild-type larvae that are absent in mutant larvae on immunoblots of agarose isoelectric focusing gels and no other protein bands are visible. Since the NDP kinase reaction occurs via a ping-pong mechanism utilizing a phosphorylated enzyme intermediate, it was hypothesized that the multiple bands detected on agarose isoelectric focusing gels represented phosphorylated intermediates of AWD/NDP kinase. To test this hypothesis, purified recombinant AWD/NDP kinase was incubated with increasing concentrations of dCTP as a phosphate donor, fractionated by agarose isoelectric focusing and immunoblotted with HA1. With increasing concentration of dCTP, the

number of bands increased to a total of seven major bands. The pI of the most basic major band was 8.3. The acidic modification caused by incubation with dCTP was interpreted as due to phosphorylation of the protein. Since no protein kinase was added to the reaction and since the substrate, dCTP, is unlikely to be used by a protein kinase, the most likely site of phosphorylation is the active-site histidine. According to this interpretation, such phosphorylated forms represent enzymic intermediates. If so, then subsequent incubation with a nucleoside diphosphate should cause transfer of phosphate from the enzyme and loss of acidic forms of the enzyme (Bominaar *et al.*, 1994). That result is, indeed, what is observed (Timmons *et al.*, 1995). It is extremely significant that when recombinant protein is incubated with high concentrations of NTP, seven isoelectric species are observed. Since, *Drosophila* NDP kinase is a hexamer (Lascu *et al.*, 1992; Chiadmi *et al.*, 1993), it was proposed that the seven isoelectric species represent hexametric, of which 0–6 of the subunits are phosphorylated. Bominaar *et al.* (1994) have investigated the autophosphorylation of *Dictyostelium* NDP kinase and concluded that essentially all of the phosphorylation of *Dictyostelium* NDP kinase represents enzymic intermediates. This interpretation is identical to the one for purified *Drosophila* NDP kinase. MacDonald *et al.* (1993) and Munoz-Dorado *et al.* (1993), on the other hand, have presented evidence for autophosphorylation on Ser residues of human NM23-H1/NDP kinase A and *Myxococcus* NDP kinase, respectively. If autophosphorylation occurs on Ser residues of purified recombinant *Drosophila* AWD/NDP kinase, it occurs at a level below the limits of detection. Extracts of wild-type larvae, viable *pn* mutant larvae, viable *awd^{Kpn}* mutant larvae and lethal *pn;awd^{Kpn}/awd^+* mutant larvae were fractionated by agarose isoelectric focusing and immunoblotted. The relative intensities of the multiple bands from mutant larvae is comparable to the relative intensities of the bands from wild-type larvae (Timmons *et al.*, 1995). Neither the *pn* mutation nor the *awd^{Kpn}* mutation has any effect on these phosphorylations. This result for *awd^{Kpn}* does not support the hypothesis that the *awd^{Kpn}* mutation affects the folding/assembly pathway. That hypothesis was based on the lower stability to heat and urea denaturation of the purified KPN protein (Lascu *et al.*, 1992). Mutational analysis suggests instead that the KPN protein has altered substrate specificity.

HOW DO SPECIFIC MUTATIONS AFFECT THE BIOLOGICAL FUNCTION OF AWD/ NDP KINASE?

Once it became clear that *Kpn* is a mutant allele of *awd*, the possibility arose of obtaining additional mutation in the *awd* gene by mutagenizing *awd^{Kpn}* male flies, mating them to *prune* females, and selecting for viable males, which would be revertants of *awd^{Kpn}*. In one report, twelve *awd* mutations were recovered, at a frequency of 1/15,000 chromosomes tested, as ethyl methanesulfonate-induced revertants of the gain of function allele, *awd^{Kpn}* (Timmons *et al.*, 1995). All of the revertants behave developmentally as loss-of-function alleles of *awd* and all of them have significantly less NDP kinase activity than the wild-type. Southern blots of genomic DNA extracted from revertant hemizygous larvae revealed that three of the alleles had deletions of the *awd* gene. One of these deletions, *awd^{KRs6}*, removes 788 base pairs, including the entire coding region of *awd* without disturbing either flanking gene. It serves as the standard for a complete *null* allele. Each of the nine other revertants revealed single amino acid changes. In two of the revertants, *awd^{KRm2}* and *awd^{KRm8}*, the initiator methionine is changed to lysine and valine, respectively. As expected from the loss of an initiator methionine, neither *awd^{KRm2}* nor *awd^{KRm8}* accumulates AWD subunits. However, in the remaining seven revertants, AWD subunits that are defective in enzyme activity do accumulate (Timmons *et al.*, 1995). These data show that the dominant lethality caused by *awd^{Kpn}* mutant subunits of AWD/NDP kinase can be reverted either by preventing the accumulation of *awd^{Kpn}* subunits or by reducing the enzyme activity of the *awd^{Kpn}* subunits.

The seven revertants recovered that accumulate enzymically inactive or less active AWD subunits affect six different conserved residues. Two of those residues, Arg89 and Arg106 hydrogen bond to the β phosphate of bound nucleotides (Williams *et al.*, 1993; Chiadmi *et al.*, 1993; Morera *et al.*, 1994). One of the residues, Glu130, positions the active site His119 (Williams *et al.*, 1993; Chiadmi *et al.*, 1993; Morera *et al.*, 1994). Tepper *et al.* (1994) have shown by site-directed mutation of the *Dictyostelium*-soluble NDP kinase that alteration of any of these three residues dramatically reduces enzyme activity, in complete agreement with these results. Based on the lack or reduction of enzyme activity in the other revertants, it has been proposed that they also disrupt substrate binding and/or catalysis. This suggests the hypothesis

that the conditional dominant lethality of *awd*[Kpn] depends on altered substrate specificity of AWD/NDP kinase subunits carrying the Pro97Ser mutation. In a *prune*[+] background, this does not cause any problem, because there is no accumulation of potentially harmful abnormal substrates. However, in a loss of function *prune* mutant a substrate accumulates that can be phosphorylated by KPN mutant subunits of AWD/NDP kinase, but cannot be acted upon by wild-type AWD/NDP kinase. The dark eye color of *prune* mutant adults is due to loss of pteridine eye pigments, which are derived from GTP. Hackstein (1992) has reported altered metabolism of guanosine injected into *prune* mutants. One of these metabolites could represent the hypothetical substrate proposed to accumulate in *prune* mutants. The inability of wild-type NDP kinase to act upon this hypothetical substrate explains why loss of *prune* function is not lethal. The Kpn loop defined by Dumas *et al.* (1992) positions the nucleotide binding cleft and, indeed, moves substantially upon substrate binding (Williams *et al.*, 1993; Chiadmi *et al.*, 1993; Morera *et al.*, 1994). According to this interpretation, the *awd*[Kpn] mutation, Pro97Ser, by virtue of altering the nucleotide binding cleft, allows the binding, in addition to normal NDPs and NTPs, of a molecule that accumulates in *prune* mutant larvae. The lethality is due to harmful effects caused by the phosphorylation of this molecule that accumulates in *prune* mutant larvae but not in *prune*[+] larvae (Timmons and Shearn, 1997).

AWD MAY NOT BE THE ONLY GENE OF *Drosophila* THAT ENCODES AN NDP KINASE

The NDP kinase specific activity in extracts of *awd* null mutant larvae is <2% of the activity in non-mutant larvae (Biggs *et al.*, 1990). This demonstrates that the *awd* gene is responsible for nearly all of the NDP kinase activity in third instar larvae. There may be other genes which provides the rest of the NDP kinase activity in third instar larvae. The product of one of these potential other genes, *nmdyn-D7*, was found by screening the *Drosophila melanogaster* EST database with the sequence of human nm23-H7 (Mehus, Johnson, and Lambeth, unpublished). The predicted gene that encodes this cDNA was identified in the *Drosophila* genomic sequence as CG 8362, which is included in AC008315. Another predicted NDP kinase gene is CG 5310, which is included in AC015210. The product of this predicted gene resembles human Nm23-H6.

FUTURE WORK

There is quite a lot known about the phenotype of *awd* gene mutations and about the structure and enzymic properties of AWD/NDP kinase. The challenge for the future will be to discover the biological role or roles of AWD/NDP kinase.

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