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Review

Analysis of glycolytic enzyme co-localization in *Drosophila* flight muscle

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Summary

In *Drosophila* flight muscles, glycolytic enzymes are co-localized along sarcomeres at M-lines and Z-discs and co-localization is required for normal flight. We have extended our analysis of this phenomenon to include a set of six glycolytic enzymes that catalyze consecutive reactions along the glycolytic pathway: aldolase, glycerol-3-phosphate dehydrogenase (GPDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), triose phosphate isomerase, phosphoglycerate kinase and phosphoglycerol mutase (PGLYM). Each of these enzymes has an identical pattern of localization. In mutants null for GPDH, localization of none of the other enzymes occurs and therefore is interdependent. In optimally fixed preparations of myofibrils, accumulation of the enzymes at M-lines is much greater than at Z-discs. However, localization at M-lines is more labile, as shown by loss of localization when fixation is delayed. We have begun to analyze the protein–protein interaction involved in glycolytic enzyme co-localization using the yeast two-hybrid system. We have identified two pair-wise interactions. One is between GPDH and GAPDH and another is between GPDH and PGLYM.

Key words: glycolytic enzyme, co-localization, *Drosophila*, flight muscle, myofibril, protein–protein interaction, M-line, Z-disc, glycerol-3-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerol mutase.

Introduction

The issue of whether functionally related enzymes are specifically organized within cells has been fraught with controversy for many years. In some cases, associations between enzymes from the same pathway have been observed, and, in recent years, the number of documented examples has increased. However, it is difficult to assess the functional significance of these enzyme–enzyme associations (Ovadi and Srere, 2000; Srere, 1987). Indeed, some have argued that these associations may not have any functional significance. The debate has focused on whether enzyme–enzyme associations along a pathway increase the flux of metabolites through the pathway. Some have argued that diffusion rates of substrate molecules are sufficiently high and the distances within a cell sufficiently small so that rates of catalysis would be unaffected by the juxtaposition of enzymes along a pathway (Wu et al., 1991, 1992). Others have suggested that these associations may have a role in establishing pool compartmentation. In any case, numerous examples clearly show that enzyme–enzyme associations occur and the focus of work now should be on testing hypotheses related to their functional significance.

Several years ago, we attempted to bring the power of genetic analysis, both classical and molecular, to extend our understanding of the functional significance of co-localized enzymes of a pathway. We initiated studies using the well-characterized glycolytic pathway in *Drosophila* and we chose adult flight musculature as a tissue for study. Muscle has well defined cytoarchitecture and, more importantly, offers the possibility of analyzing the functional importance of enzyme interactions using the selectable phenotype flight as a marker of genetic perturbation.

Studies were begun using the glycolytic enzyme glycerol-3-phosphate dehydrogenase (GPDH). This enzyme and its gene (*gpdh*) have been well characterized (Kotarski et al., 1983; MacIntyre and Davis, 1987; von Kalm et al., 1989). A large number of mutant alleles at this locus have been isolated and some result in the loss of flight ability. When flight muscle was analyzed using immunofluorescence microscopy, GPDH was found localized at Z-discs and M-lines. When immune serum was used to react with sections of thoraces of GPDH null mutants that are known not to synthesize any immunologically cross-reactive material, no specific labeling of myofibrils was detected. Localization of two other glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase, was also detected in flight muscle at Z-discs and M-lines, in a manner indistinguishable from that of GPDH. The striking result was that in GPDH null mutants, GAPDH and aldolase are not properly

localized at Z-discs or M-lines but are found instead in the extramyofibrillar spaces (Wojtas et al., 1997).

When molecular characterization of the *gpdh* gene and its expression was completed (von Kalm et al., 1989), it was determined that this gene encodes three isoforms produced through differential splicing of three exons at its 3′ end. One of these, GPDH-1, is found only in thoracic musculature and is the only isoform found in this tissue. The tissue specificity of this isoform offered a unique opportunity to generate genetically engineered flies that could make the other isoforms but not GPDH-1. Significantly, GPDH-1, as compared with the other two isoforms, has a C-terminal tripeptide, Q-N-L. This tripeptide is encoded by a specific exon, exon 8, that is separated from the rest of the gene by a 1-kb intron.

We then set out to determine if GPDH-1 localization is accomplished, at least in part, through its C-terminal tripeptide. Transgenic flies that produce only GPDH-3 were made (Wojtas et al., 1997). This isoform is distinguished from GPDH-1 by lacking the C-terminal tripeptide and is not normally produced in muscle. In these transgenic flies, the endogenous *gpdh* promoter was used to drive GPDH-3 production, and the resident *gpdh* locus was homozygous for alleles unable to produce GPDH. We found that in flight muscle of these engineered flies, GPDH-3 is not localized at Z-discs and M-lines but is found in the extramyofibrillar spaces. As is the case in GPDH null mutants, GAPDH and aldolase are also not localized at Z-discs and M-lines in these engineered flies.

Once we established that the glycolytic enzymes are localized in an interdependent manner, we next wished to determine if this localization has functional significance. We found that genetically engineered flies that produce only GPDH-3 in their flight muscles cannot fly normally even though they have appreciable GPDH activity in their flight muscles. Therefore, the interdependent co-localization of glycolytic enzymes, and not simply their activity in flight muscle cells, is required for flight.

A long-term goal is to identify protein–protein interactions that support glycolytic enzyme localization. In this paper, we present the analysis of an expanded set of glycolytic enzymes and more precisely examine details of glycolytic enzyme localization at M-lines and Z-discs to determine if enzyme binding at the two sites has different properties. We have begun to identify some of the pairwise protein–protein interactions that may be involved in myofibrillar glycolytic enzyme co-localization.

Materials and methods

Drosophila *strains*

Drosophila melanogaster Oregon-R is the wild-type strain used in these studies. GPDH null mutant combinations were made using three different chromosomes. The JH231 chromosome carries an insertion of a transposable element insertion in exon 6 of the *gpdh* gene and does not produce any GPDH transcripts (D. T. Sullivan, N. Fuda and R. J. MacIntyre, unpublished). Df(2L)clot-7 is a cytogenetically visible deficiency that spans the *gpdh* locus. The mutation *nsp10* is a nonsense mutation in codon 12 (D. T. Sullivan, N. Fuda and R. J. MacIntyre, unpublished). Crosses to construct the null strains used here generated combinations of two of these chromosomes. Each mutation results in the loss of immunologically reactive material.

Myofibril preparation

Myofibrils were prepared from adults of either sex that were 2–6 days post emergence. Flies were immobilized by chilling, and heads and abdomens were separated from the thorax. Thoraces were opened in fixative (see below) and dissected free of non-muscle material; fixation was for 60 min on ice. The fixative comprised 4% paraformaldehyde, 0.5% glutaraldehyde, 0.1 mol l^{-1} phosphate buffer, pH 7.2. Following fixation, the thoraces were homogenized, and myofibrils were collected on Transwell polyester membrane filters (12 mm diameter, 3.0 μm pore size; Corning) and then washed with three changes of 0.5 mol l^{-1} phosphate, 0.15 mol l^{-1} NaCl, 0.1 mol l^{-1} Tris. The filters were incubated with primary antibodies for 1 h, washed with three changes of phosphate buffer saline (PBS) containing 0.1 mol l^{-1} Tris, pH 7.2. The filters were then incubated with non-immune goat serum (Pierce) as a blocking agent for 15 min and then incubated with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit serum. The filters were washed three times in PBS-Tris. The filters were cut out from their frame and mounted on a slide for fluorescence microscopy.

Antibodies

Antibodies against *Drosophila* GPDH and GAPDH were generated by immunizing rabbits with proteins purified from flies as previously described (Skuse and Sullivan, 1985; Sullivan et al., 1985). Antibodies against *Drosophila* aldolase (Ald), triose phosphate isomerase (TPI), phosphoglycerate kinase (PGK) and phosphoglycerol mutase (PGLYM) were generated by cloning full-length cDNA coding for each protein into the vector pTrcHis (Invitrogen). The fusion protein was isolated and the sample sent to Josman Laboratories, CA, USA for immunization of rabbits and antisera preparation. Each serum was used at the maximal dilution possible to obtain an adequate signal; in each case, the concentration was less than or equal to a 1:1000 dilution.

Visualization of actin

Actin was visualized by staining with rhodamine-conjugated phalloidin. Myofibrils were mounted in glycerol and viewed using a 100× 1.3 NA objective mounted on a Nikon TE-300 microscope fitted for epifluorescence. Excitation and emission of rhodamine and FITC were accomplished with Texas red and FITC optimized filter sets (Nikon), respectively. Images were captured with a MicroMax 5 MHz CCD camera (Roper/Princeton Instruments).

Molecular biology

Molecular biological techniques were performed using

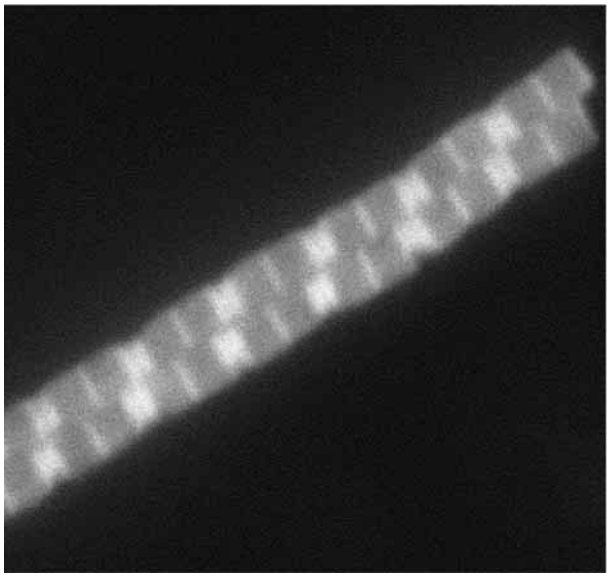

Fig. 1. Glycerol-3-phosphate dehydrogenase (GPDH) localization in antibody-stained myofibrils. Myofibrils were isolated from wild-type *Drosophila* muscles and reacted with anti-GPDH serum and then reacted with fluorescein isothiocyanate-labeled goat anti-rabbit serum and visualized as described in Materials and methods.

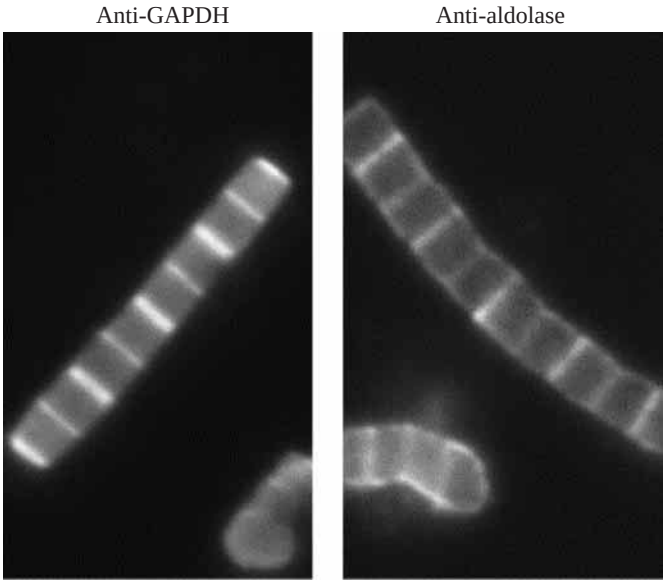

Fig. 2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase localization in antibody-stained myofibrils, as in Fig. 1.

standard procedures (Maniatis et al., 1982). The yeast two-hybrid system was used as described (Bai and Elledge, 1997) with the plasmids pAct II and Pas-I. Yeast cells containing the desired pair of plasmids were generated by co-transformation and selection for leu^+,trp^+ cells. Interaction was tested by selecting for leu^+,trp^+,his^+ cells in media supplemented with 25 mmol l^{-1} 3-amino triazole to assess activation of the *his* promoter. Spot tests were then done to verify activation of the *β-gal* promoter. Cells demonstrating transcription from both *his* and *β-gal* promoters were grown in liquid culture and assayed fluorimetrically for β-galactosidase using the FluorAce reporter assay kit (BioRad).

Results

We have previously shown subcellular localization of three glycolytic enzymes, GPDH, GAPDH and aldolase, in flight muscle cells. In these studies, localization was visualized using fluorescent microscopy of sections of fixed thoraces reacted with anti-GPDH, anti-GAPDH or anti-aldolase followed by reaction with FITC-labeled goat anti-rabbit serum and visualized using fluorescent microscopy. In the flight muscle, immune-reactive material can be seen in a pattern along the sarcomeres that correspond to M-lines and Z-discs. We demonstrated the specificity of immunofluorescence localization in two ways. First, GPDH staining was not found using non-immune serum. We have used pre-immune sera as controls for the other antisera used here and also have found no M-line or Z-disc labeling. Second, sections of muscle from GPDH null mutants, which do not make GPDH, show no immunolocalization when reacted with anti-GPDH serum (Wojtas et al., 1997).

In isolated myofibrils, M-lines and Z-discs show a pattern of GPDH fluorescence labeling similar to that of sectioned thoraces, but it often appears that M-lines and Z-discs have an alternating pattern of bands with different relative intensities (Fig. 1). This pattern is sometimes seen in sectioned material but the difference between alternating bands is not as striking. In the isolated myofibrils shown in Fig. 1, it is apparent that the brighter band appears to be interrupted centrally, i.e. the band appears as a doublet. We have extended our observations on using isolated myofibrils to determine whether other glycolytic enzymes are similarly localized to those previously studied and to determine whether the variable M-line–Z-disc intensity pattern might provide a suggestion as to the mechanisms of glycolytic enzyme localization in the myofibril.

A similar pattern of localization of GAPDH and aldolase at M-lines and Z-discs is also apparent in isolated myofibrils (Fig. 2); the same is true for TPI, PGK and PGLYM (Fig. 3). Each enzyme has a similar pattern of localization, i.e. alternating bright and less-intense bands. The brighter band appears to be bisected by a narrow line of reduced or no antibody binding. Using antisera against PGK, we find the same alternating bands with different fluorescent intensity. However, the brighter band does not show the central dark zone. We do not know whether this represents a real difference in PGK localization or is due to technical issues. We have found in all our studies, both immunofluorescence microscopy and western blotting, that levels of anti-PGK-reactive material in flight muscle are much lower than for the other glycolytic enzymes we have studied. In addition, we sometimes find when using the other antisera that the central dark band is not seen in sarcomeres that appear weakly fluorescent, possibly due to inadequate fixation. However, the central point is that six glycolytic enzymes that catalyze consecutive reactions along

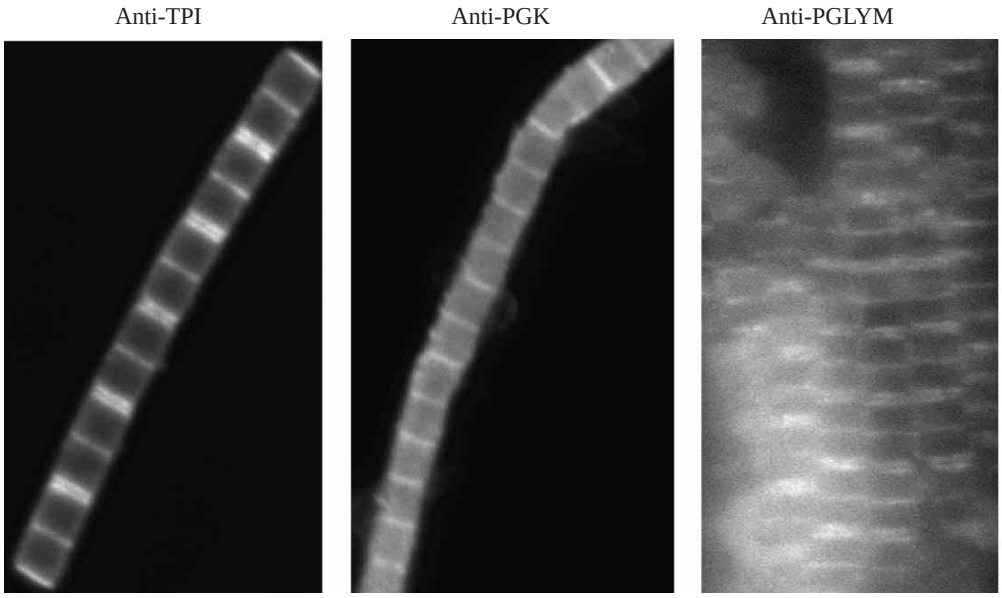

Fig. 3. Triose phosphate isomerase (TPI), phosphoglycerate kinase (PGK) and phosphoglycerol mutase (PGLYM) localization in antibody-stained myofibrils, as in Fig. 1.

the glycolytic pathway are found similarly localized in flight muscles at M-lines and Z-discs.

To determine whether M-lines or Z-discs are represented by the more brightly fluorescing bands, we conducted a double label experiment using FITC-labeled anti-GPDH and rhodamine-labeled phalloidin. Results of these experiments are shown in Fig. 4. Phalloidin binds to actin and its staining pattern shows labeling along the A-band, as expected. There is an alternating pattern of darker phalloidin-unlabeled bands. We interpret the narrow bands as representing Z-discs and the broader bands as representing M-lines. Phalloidin binds to actin and, since actin does not cross the area of the M-line but does penetrate into the Z-disc, our expectation is that the actin fluorescent pattern will show broader M-lines and narrower Z-discs and be therefore distinguishable. Comparing the GPDH pattern to the phalloidin pattern reveals that the bright GPDH bands correspond to the broader non-phalloidin-labeled M-lines, and the less intensely anti-GPDH-labeled bands are the Z-discs.

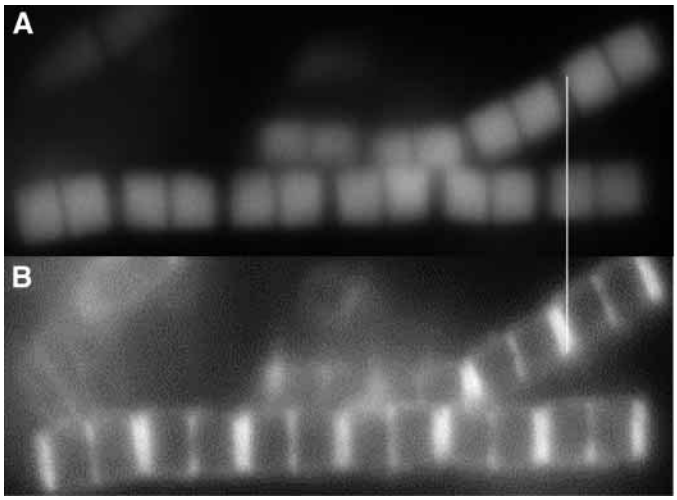

Fig. 4. A comparison between (A) phalloidin and (B) anti-glycerol-3-phosphate dehydrogenase (GPDH) doubly stained myofibrils. Note that the thin Z-discs and the broader M-lines in the phalloidin-stained myofibril coincide with fainter anti-GPDH stained and brighter stained corresponding bands, respectively.

We have also found that localization of the glycolytic enzymes at M-lines is more labile that at Z-discs. In developing procedures for the preparation of myofibrils, it became apparent that rapid fixation is required to retain the maximal M-line–Z-disc labeling pattern. We have compared the intensity of fluorescence in muscles dissected in fixative with that found in muscles dissected in buffers, incubated and then transferred to fixative. The fluorescence intensity at M-lines can be lost or much reduced, as shown in Figs 5, 6, if muscles are not dissected directly in fixative. Thoracic muscles were dissected in contraction buffer (100 mmol l^{-1} KCl, 5 mmol l^{-1} KPO_4, 4 mmol l^{-1} $CaCl_2$, 5 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} ATP, pH 7.0) and incubated on ice in contraction buffer for 5 min (Fig. 5) or for 20 min (Fig. 6A). They were then fixed and examined for GPDH localization. The fluorescent pattern is greatly diminished at M-lines and in some cases disappears completely. The Z-disc fluorescence is minimally affected. Comparison of Fig. 6A with Fig. 6B (myofibrils simultaneously labeled with phalloidin) confirms that it is the M-line that loses anti-GPDH labeling. In contraction buffer, M-lines appear narrower, as would be expected in muscles put under contraction conditions. The loss of M-line localization also occurs in relaxation buffer (data not shown), showing that it is the delay in fixation, not the act of contraction, that results in the loss of glycolytic enzymes from the M-line. Therefore, during preparation, the signal from M-lines, which is more intense than from Z-discs, in rapidly fixed myofibrils, is more easily perturbed than the signal from Z-discs. This implies that

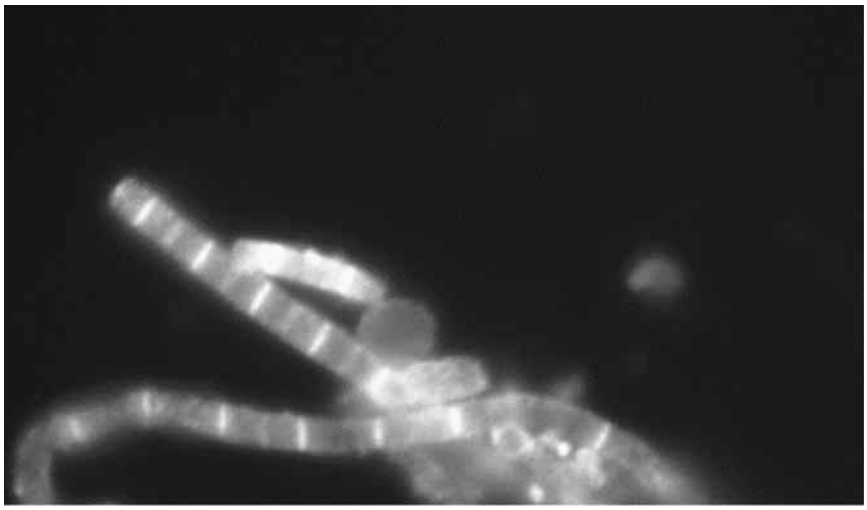

Fig. 5. Myofibrils from wild-type *Drosophila* muscles incubated for 5 min in contraction buffer and stained for glycerol-3-phosphate dehydrogenase (GPDH). Note the reduced staining at the M-lines as compared with Fig. 1.

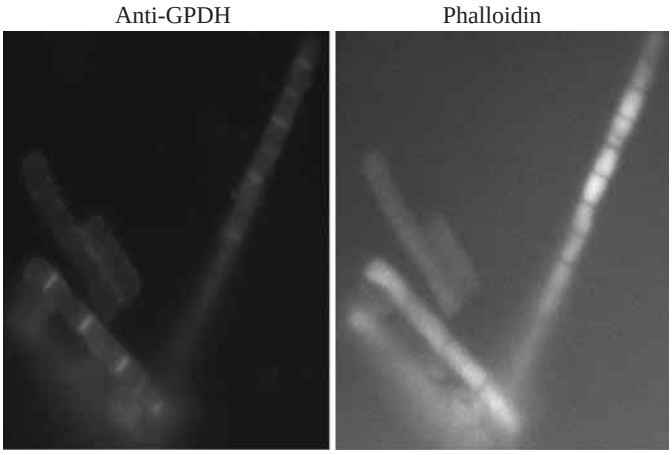

Fig. 6. Myofibrils from wild-type *Drosophila* muscles incubated in contraction buffer for 20 min and simultaneously stained using anti-glycerol-3-phosphate dehydrogenase (GPDH) and phalloidin. Note the correspondence of the brightly fluorescent GPDH signal with the very faint, narrow dark bands on the phalloidin-stained myofibril.

the organization or binding of glycolytic enzymes at M-lines and Z-discs is different and that glycolytic enzymes are bound less tightly at M-lines than at Z-discs.

We have reported (Wojtas et al., 1997), using sections of flight muscles, that sarcomeric localization of GAPDH and aldolase does not occur in the absence of proper GPDH localization. This is the case using GPDH null mutants or in genetically engineered flies that ectopically produce only the GPDH-3 isoform in muscle cells.

Similar results are obtained using preparations of myofibrils. Myofibrils prepared from GPDH null mutants stained with anti-GPDH show no immunoreactive material when stained with anti-GPDH, confirming the specificity of the immunofluorescence microscopy (Fig. 7). Neither GAPDH or aldolase (Fig.·7) nor TPI, PGK or PGLYM (Fig. 8) are found localized at M-lines or Z-discs in myofibrils isolated from GPDH null mutants. Therefore, all glycolytic enzymes that have been analyzed to date behave as a set and are similarly and interdependently localized.

We have initiated several biochemical and genetic approaches to identify those protein–protein interactions that are functionally important for glycolytic enzyme co-localization. These would include interactions that occur among members of the set of glycolytic enzymes and interactions between the glycolytic enzymes and other muscle structural proteins. Initial results on interactions among the glycolytic enzymes have been obtained using the yeast two-hybrid assay system. cDNAs for each of the glycolytic enzymes – PFK, aldolase, GPDH, GAPDH, TPI, PGK and PGLYM – were introduced into the vectors pAS1 and pACT2 and then transformed into yeast cells of both a and α mating types. Yeast matings were conducted in all pairwise combinations where each of the parental plasmids contained a different member of the set of seven glycolytic enzymes. Cells of the genotype his^+,leu^+,trp^+ were selected and tested for β-galactosidase expression. Those testing positive for expression from both *his* and *β-gal* promoters were then grown in liquid culture, and β-galactosidase activity was measured fluorimetrically. Whereas most pairs of glycolytic enzymes failed to show evidence of interaction, two glycolytic pairs – GPDH/GAPDH and GPDH/PGLYM – did show activation of both promoters, suggesting that these proteins can interact in yeast cells. The cells containing these plasmid pairs and the activities of several control plasmid combinations in terms of units of β-galactosidase activity are reported in Table 1.

Subunits of GPDH-1 and GPDH-3 do interact, as might be expected, since GPDH is an active dimer. Subunit interaction is also seen in the Ald/Ald combination, also expected since aldolase has a homotetrameric structure. However, we see no evidence of aldolase interactions with either GPDH-1 or with GAPDH. We also fail to see evidence of any interactions when using TPI, PGK, PGLYM or PFK in any pairwise combination with any other members of this set of glycolytic enzymes. Of note is the interaction of GPDH-3 and GAPDH. If the

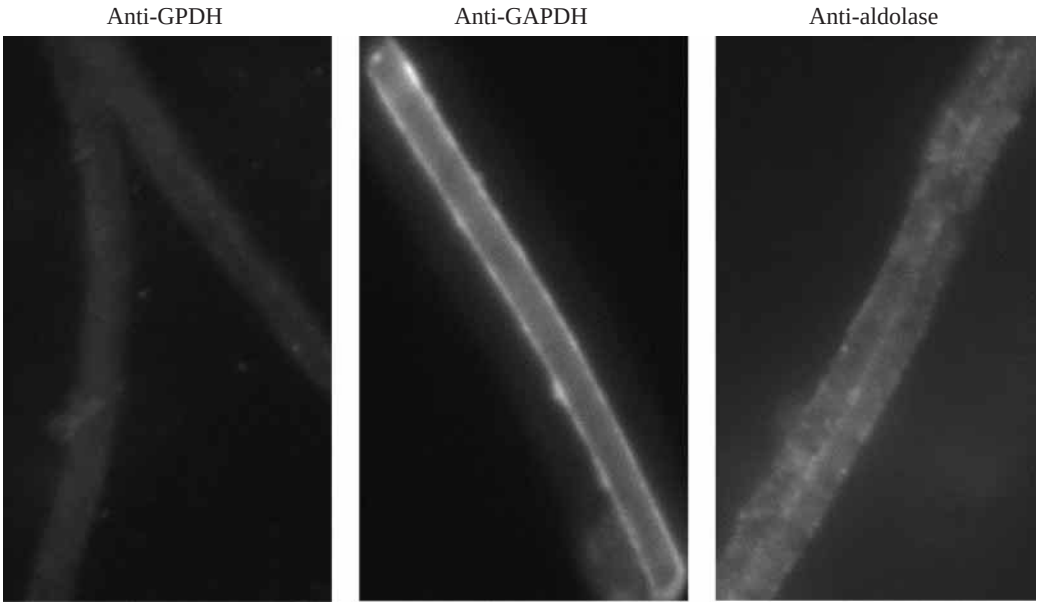

Fig. 7. Glycerol-3-phosphate dehydrogenase (GPDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase in antibody-stained myofibrils prepared from GPDH null mutant flies. Visualized as in Fig. 1. The exposure has been extended so that an image of the weakly fluorescent myofibril would be evident.

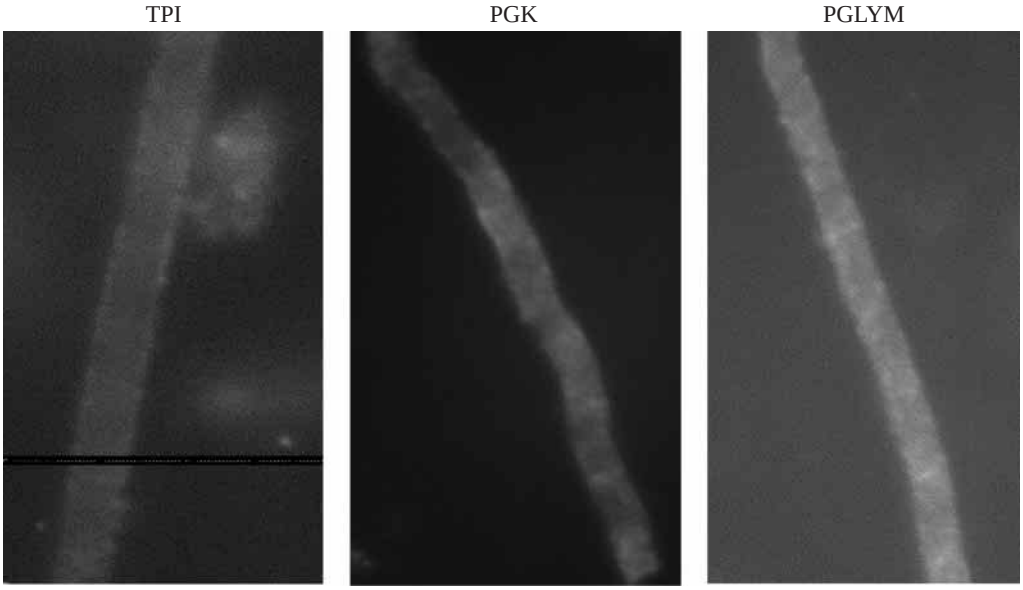

Fig. 8. Triose phosphate isomerase (TPI), phosphoglycerate kinase (PGK) and phosphoglycerol mutase (PGLYM) in antibody-stained myofibrils prepared from glycerol-3-phosphate dehydrogenase (GPDH) null mutant flies. Visualized as in Fig. 1. The exposure has been extended so that an image of weakly fluorescent myofibril would be evident.

interactions between GPDH and GAPDH seen using the yeast two-hybrid assay are reflective of an *in vivo* functionally relevant interaction, this suggests that these glycolytic interactions may occur in cells other than muscle cells, where GPDH-3 is not found.

We have attempted to identify possible targets of glycolytic binding in muscles by testing the set of glycolytic enzymes with fusion proteins of the muscle proteins. Proteins tested include flight muscle actin, actinin, troponin C and troponin I. In no cross did results establish an interaction of one of these proteins with a glycolytic enzyme. Since the yeast two-hybrid system seems most well suited to detect fairly strong protein–protein interactions, these results suggest that if interaction between any of these proteins does occur, it is likely to be weak. This conclusion also applies to the negative tests for interaction among members of the set of glycolytic enzymes.

Table 1. *β-galactosidase activity in yeast cells with plasmids pAct-II, pAs-I or derivatives*

his^+,leu^+ yeast plasmids	β-galactosidase activity
pAct-II/pAs-1	0.004
pAct-IIGpd-1/pAs-I	0.003
pAct-IIGpd-3/pAs-I	0.002
pAct-IIGpd-1/pAs-IGpd-3	0.521
pAct-IIGpd-3/pAs-IGpd-1	0.485
pAct-IIGpd-1/pAs-IGap	0.135
pAct-IIGpd-3/pAs-IGap	0.582
pAct-IIGpd-3/pAs-IPglym	0.903
pAct-IIAld/pAs-IAld	0.631
pAct-IIAld/pAs-IGpd-1	0.003
pAct-IIAld/pAs-IGap	0.007

Abbreviations: Gpd, glycerol-3-phosphate dehydrogenase; Gap, glyceraldehyde-3-phosphate dehydrogenase; Pglym, phosphoglycerol mutase; Ald, aldolase.

β-galactosidase activity is expressed in fluorescence units per mg protein in extracts of yeast cells. Each value is the mean of three determinations.

Discussion

In *Drosophila* thoracic muscle, we have determined that six glycolytic enzymes that catalyze consecutive reactions are localized at M-lines and Z-discs. Binding of each member of the set shows an identical pattern. It seems, however, that there are differences in the properties of the enzyme associations at these two sites. Using optimal dissection and fixation procedures, labeling intensity at M-lines is higher, implying a higher enzyme concentration, than at Z-discs. However, M-line labeling seems to be easily lost if fixation following dissection is delayed. Z-disc labeling, on the other hand, is more consistent, implying more-stable associations. We do not think this variation is a property of a specific thoracic muscle type because we have prepared myofibrils from specifically dissected indirect flight muscles and found that their M-line–Z-disc fluorescence is no different from that found in myofibrils prepared from entire thoraces (data not shown). Binding at glycolytic enzymes seems to be absent in the most central region of the M-line. However, in poorly fixed samples, the entire M-line appears fluorescent. We have interpreted this change to be due to diffusion of the enzymes away from their primary binding site in the M-line. We have not as yet been able to account for this result in terms of binding to a specific M-line protein. The association at the Z-discs, on the other hand, seems more stable, and fluorescence is uniform over the Z-disc. This difference is probably due to the glycolytic enzymes having associations with different partners at the two sites. It could also be reflective of a more dynamic process. We often see a slight haze of immunofluorescence along the sarcomere between the Z-discs and M-lines both in sections and in isolated myofibrils. The intensity of this haze is insufficient to be confident of its meaning but has led us to speculate on a model that might relate M-line and Z-disc localization of glycolytic enzymes to A-bands and energy utilization. Using immunofluorescent microscopy, we are visualizing this system statically and it is, of course, dynamic. It is possible that glycolytic enzyme complexes could enter the center of the sarcomere at one site, M-line or Z-disc, and then move along the length of the A-band to the other site, where they may then leave. This would bring the glycolytic enzymes in close proximity to the acto-myosin complex where ATP is used. We have no basis to propose which site, M-line or Z-disc, might be the point of entry or site of leaving. Movement along the sarcomere could be driven by muscle contraction providing hydrodynamic forces and flow. One question that arises is whether the enzymes are localized along the sarcomere as rings or whether they penetrate the structure of the sarcomere. We have begun analysis of glycolytic enzyme localization using confocal microscopy. Our results remain preliminary, but optical sections reveal that the glycolytic enzymes are found throughout the depth of the M-lines and Z-discs. Labeling is not confined to the surface.

The function of glycolysis in muscle is to provide ATP for myosin ATPase to enable contraction. This may be either directly from the phosphorylation reactions of the glycolytic pathway or by providing pyruvate for mitochondrial oxidative phosphorylation. Glycolysis presumably also plays a role, either directly or indirectly, in providing ATP for myosin phosphorylation. Since flight ability is greatly diminished when the glycolytic enzymes are not co-localized (Wojtas et al., 1997), a rather direct role of glycolytic enzyme co-localization in muscle activity is suggested. But the glycolytic enzymes, at least for the most part, are not localized at the A-band, the sites of myosin localization and ATP utilization. At present, we have insufficient understanding of the mechanisms that connect glycolytic enzyme co-localization and muscle contraction.

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References

Bai, C. and Elledge, S. (1997). The yeast two-hybrid system. In *The Yeast Two-Hybrid System* (ed. P. Bartel and S. Fields), pp. 11-28. New York: Oxford University Press.

Kotarski, M. A., Pickert, S., Leonard, D. A., LaRosa, G. J. and MacIntyre, R. J. (1983). The characterization of α-glycerophosphate dehydrogenase mutants in *Drosophila melanogaster*. *Genetics* **105**, 387-407.

MacIntyre, R. and Davis, M. (1987). A genetic and molecular analysis of α-glycerolphosphate cycle in *Drosophila melanogaster*. In *Isozymes: Current Topics in Biological Research*, vol. 14 (ed. M. Ratazzi, J. Scandalios and G. Whitt), pp. 195-224. New York: Alan R. Liss.

Maniatis, T., Fritsch, D. F. and J. Sambrook. (1982). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Ovadi, J. and Srere, P. (2000). Macromolecular compartmentation and channeling. In *Inter. Nat. Review of Cytology*, vol. 192, pp. 255-280: Academic Press.

Skuse, G. R. and Sullivan, D. T. (1985). Developmentally regulated alternate modes of expression of the Gpdh locus of Drosophila. *EMBO J.* **4**, 2275-2280.

Srere, P. A. (1987). Complexes of sequential metabolic enzymes. In *Annual*

Review of Biochemistry, vol. 56 (ed. P. Boyer, I. Dawid and A. Meister), pp. 89-124. Palo Alto: Ann. Rev. Inc.

Sullivan, D. T., Carroll, W. T., Kanik-Ennulat, C. L., Hitti, Y., Lovett, J. A. and VonKalm, L. (1985). Glyceraldehyde-3-phosphate dehydrogenase from *Drosophila melanogaster*: identification of two isozymes forms encoded by separate genes. *J. Biol. Chem.* **260**, 4345-4350.

von Kalm, L., Weaver, J., DeMarco, J., MacIntyre, R. J. and Sullivan, D. T. (1989). Structural characterization of the α-glycerol-3-phosphate dehydrogenase-encoding gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **86**, 5020-5024.

Wojtas, K., Slepecky, N., vonKalm, L. and Sullivan, D. (1997). Flight muscle function in *Drosophila* requires co-localization of glycolytic enzymes. *Mol. Biol. Cell* **8**, 1665-1675.

Wu, X. M., Gutfreund, H. and Chock, P. B. (1992). Kinetic method for differentiating mechanisms for ligand exchange reactions – application to test for substrate channeling in glycolysis. *Biochemistry* **31**, 2123-2128.

Wu, X. M., Gutfreund, H., Lakatos, S. and Chock, P. B. (1991). Substrate channeling in glycolysis: a phantom phenomenon. *Proc. Natl. Acad. Sci. USA* **88**, 497-501.