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Glycogenin from *Drosophila melanogaster* and *Ceratitis capitata.*

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There are no reports focusing on glycogen synthesis in *Drosophila* or other insects that take into account the recent advances made in yeast, nematodes, and vertebrates on this subject. The biosynthesis of glycogen in vertebrates and yeast involves an initiation phase requiring autocatalytic intramolecular glucosylation of the core dimeric protein acting as a glycogen initiator synthase, followed by a polymerization phase catalyzed by glycogen synthase (Cao *et al.*, 1995; Cheng *et al.*, 1995). The latter is associated to the initiator and gives rise to unbranched amylose chains. Glycogen formation is completed by the so-called branching enzyme, that ramifies the amylose glucan (Tolmasky and Krisman, 1987; Tolmasky *et al.,* 1998) to form mature glycogen molecules. No insect homologue of mammalian or yeast glycogenins has been biochemically characterized to date. Moreover, there are no known mutant alleles of glycogen in *Drosophila* or other insects. As soon as the complete *Drosophila* genome was published (Adams *et al.,* 2000), putative gene sequences for glycogenin, glycogen synthase, and branching enzymes (Table 1) were identified. This information opens up a number of research possibilities, particularly on the regulation of gene expression under different physiological conditions.

Table 1. Characteristics of the postulated enzymes involved in glycogen biosynthesis in *D. melanogaster*.

Enzyme	FlyBase accession number	mRNA length (bp)	Chromosome localization	Gene product (aa)	Predicted Mw (Da)
Glycogenin	FBgn0034603 Gene product: CG9480	950	2R	307	34856
Glycogen synthase	FBgn0038293 Gene product: CG6940	2520	3R	689	79235
Branching	FBgn0033801 Gene product: CG4023	2598	2R	865	99761

Partially purified enzymatic fractions of *D. melanogaster* and *C. capitata* were incubated in the presence of UDP-[^{14}C]Glc to identify glucosylating proteins. The catalytic characteristics of the insect enzymes appeared to be similar to the mammalian ones (not shown). As shown in Figure 1A, we have identified glucosylated proteins behaving as glycogen initiators that seem closely related in size to other metazoan glycogenins. Based on data from SDS-PAGE, the apparent size of the putative *Drosophila* glycogenin seems smaller than that of mammalian glycogenin, whereas that of the *C. capitata* glycogenin-like protein seems similar to mammalian glycogenin (Figure 1A). Pulse-chase experiments show that these glycogenin-like molecules increase in size when further incubated with UDP-Glc (Figure 1 B). Moreover, when the core of glycogen particles was isolated and labeled with ^{125}I, a protein with a similar apparent Mw (37-47 kDa) was detected. The predicted protein

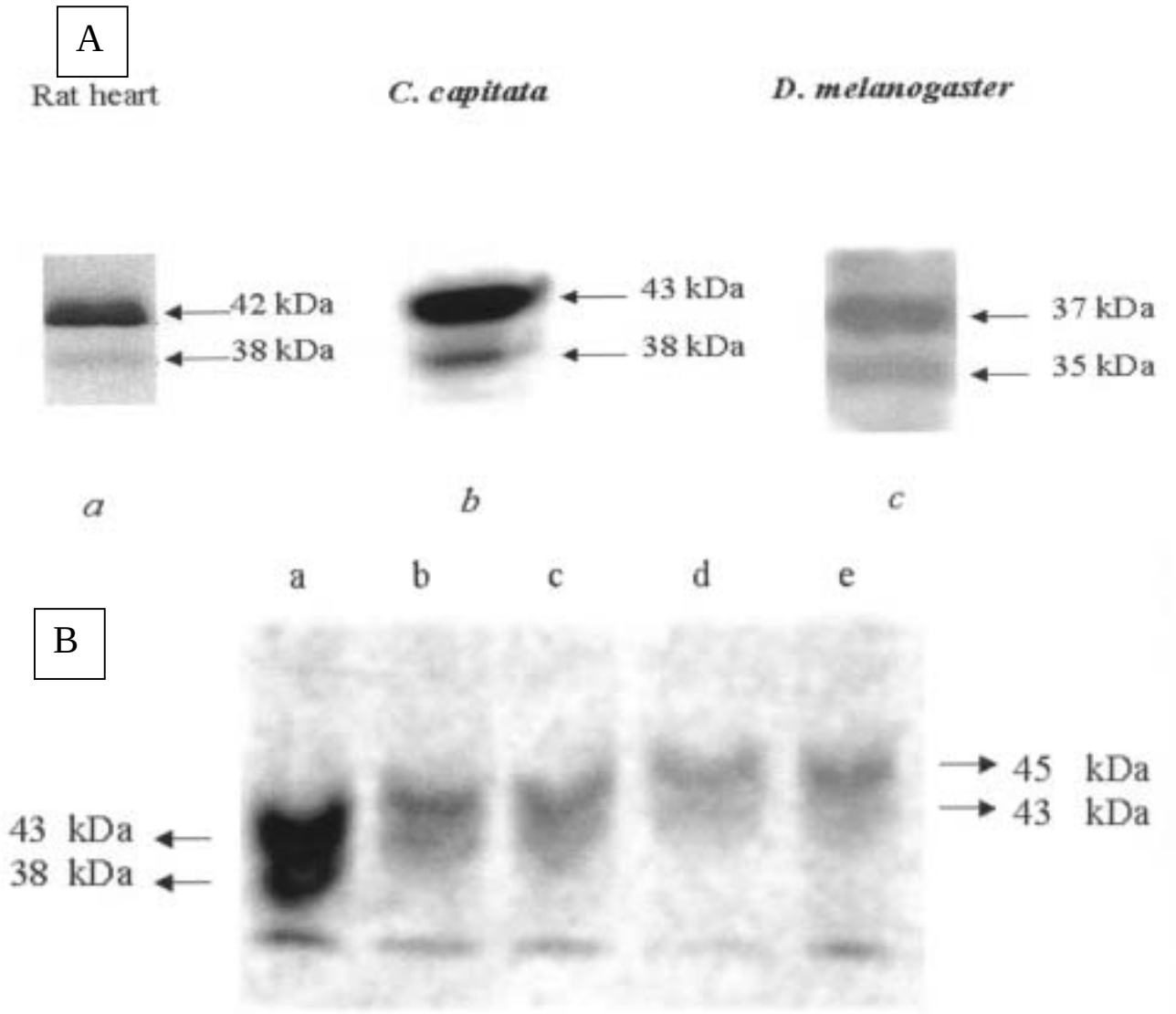

Figure 1. Characterization of *D. melanogaster* glycogenin. Autoradiography of (A): glycogen initiator proteins, glycogenins, from *D. melanogaster* (*c*) and *C. capitata* (*b*) in comparison with rat heart glycogenin (*a*). (B): Pulse-chase experiment from *C. capitata* glycogenin. The samples were incubated for 30 min in the presence of 14 μM UDP-(^{14}C)Glc. Subsequently, 10 mM UDP-Glc plus 10 mM Glc-6-P were added and incubated for different periods; 0 min (lane a), 15 min (lane b), 30 min (lane c), 45 min (lane d), and 60 min (lane e). The apparent molecular masses are indicated with arrows.

sequence of *D. melanogaster* glycogenin should have 307 amino acids sharing 57% identity and 72% positivity with mammalian glycogenins (rabbit, mouse, rat, human).

Figure 2 shows the analysis of conserved sequences shared by all the glycogenin proteins from mammals (Roach and Skurat, 1997) and *D. melanogaster*. Most important, Lys 85, which is postulated to interact with the phosphate present in the substrate UDP-Glc, is conserved in the predicted *D. melanogaster* glycogenin (Figure 2). Near Lys 85, there is a D-X-D motif (Figure 2, position 101-103) that is implicated in Mn^{+2} binding (Mn^{+2} is a requirement for glycogenin activity) which is also

conserved among glycogenins. An imperfect Leu zipper including Lys 85, which is probably involved in protein-protein interactions, is also present in the *D. melanogaster* glycogenin (Figure 2, Leu 72, 74, 80, 86, 91). The autoglucosylating activity of glycogenins (Roach and Skurat, 1997) occurs through the attachment of the first glucose to Tyr 194 by a glucose-O-tyrosil linkage. This first glucose residue is bound to the subsequent glucose residues by α1,4-glucosydic linkage. Then, polymerization continues, synthesizing an α1,4-glucan bound to protein. Tyr 194 and the amino acids flanking it which are conserved in mammalian and yaest glycogenins are also present in *D. melanogaster* glycogenin (Figure 2).

We can conclude from these results that the *D. melanogaster* and *C. capitata* glucosylated proteins described here are probably self-glucosylating proteins, similar to those demonstrated in yeast and mammals, giving rise to insect glycogen particles. This is the first time such insect proteins with the attributes of glycogenin have been biochemically characterized and these are the first invertebrate glycogenins described to date.

Figure 2. Alignment of primary sequences of *D. melanogaster* and rabbit muscle glycogenins. Protein sequences of glycogenin were analyzed using the BLAST Sequence program: (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences were compared using the Align program: (http://www.molbiol.soton.ac.uk/compute/align.html). Black boxes indicate amino acid identity.

Materials and Methods

Preparation of homogenates

Crude extracts of insects were prepared using batches of N_2^{liq}-frozen flies that were homogenized with 50 mM glycine/NaOH buffer, pH 8.6 containing 5 mM EDTA, 5 mM 2-

mercaptoethanol, 12 μM E64 and 1 mM PMSF (buffer A). After 20 strokes in a teflon-glass tissue grinder, the homogenates were centrifuged at 25,000 ×*g* for 30 min at 4ºC. The supernatant was centrifuged at 150,000 ×*g* for 2 h at 4ºC and the resulting supernatant (S_{150}) was used as a source of enzymes.

Glucosylation of glycogenin

Glucose incorporation into glycogenin was measured as described by Tolmasky *et al*. (1998). Incubation mixtures contained 50 mM Tris-HCl pH 7.5, 10 mM DTT, 6 mM $MnCl_2$, 10μM UDP-[^{14}C]Glc (700 cpm/pmol), and 0.1 mg of enzyme protein, in a total volume of 50 μl. After incubation for 30 min at 37ºC, the reactions were stopped by the addition of cracking buffer and the samples were analyzed by PAGE/SDS as described by Laemmli on 10% (w/v) acrylamide resolving gel with a 3% stacking gel.

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