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Iron availability dramatically alters the distribution of ferritin subunit messages in *Drosophila melanogaster*

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ABSTRACT **Insect ferritins have subunits homologous to the heavy and light chains of vertebrate ferritins. Cloning and sequence of the heavy chain homologue (HCH) of *Drosophila melanogaster* ferritin subunit have been reported earlier. When Northern blots of *D. melanogaster* RNA were probed with a cDNA for this HCH, three bands were observed. It was shown that these represented at least four classes of mRNA of various lengths. The polymorphism results from alternative splicing of an intron in the 5′ untranslated region (UTR) that contains the iron-responsive element (IRE) and from two alternative polyadenylation sites in the 3′ UTR. This has also been reported by others [Lind, M. I., Ekengren, S., Melefors, Ö. & Söderhäll, K. (1998) *FEBS Lett*. 436, 476–482]. By hybridizing Northern blots with specific probes, it has been shown that the relative proportions of the messages vary with the life stage and especially with iron supplementation of the diet. Iron significantly increases the amount of ferritin HCH messages and dramatically shifts the balance toward those messages that lack an IRE and/or have a short 3′ UTR. In the larvae this change takes place in the gut, but not in the fat body. We speculate that this dramatic shift in message distribution may result from an effect of iron on the rate of transcription or message degradation, or from an effect on the splicing process itself. Synthesis of ferritin HCH subunit mRNAs that lack an IRE may be important under conditions of iron overload.**

Ferritins are large spherical molecules that are found in nearly all bacteria, plants, and animals. In most cases, they are cytoplasmic proteins that serve to store ferric ions. Insect ferritins, however, differ from those of vertebrates in several respects. They have a larger size, the result of being composed of larger subunits, and significantly, they are secreted from cells and taken up by cells (1–3). Sequences of ferritin subunits from several insect species have been determined. It is clear that smaller subunits of the insect ferritins are the homologues of the vertebrate heavy chains, whereas the larger insect ferritin subunits are homologues of the vertebrate light chains. We have suggested that the sequence differences that set insect ferritin subunits apart from both those of vertebrates and of plants are a reflection of a quite different function in the case of the insects (4). It is not yet clear exactly what that function is.

The control of synthesis of ferritin subunits in vertebrate cells is frequently at the translational level. Ferritin mRNAs contain a stem loop region (iron-responsive element, IRE) in the 5′ untranslated region (UTR) that can bind a trans-acting element, the iron regulatory protein (IRP), which prevents translation of the message. The IRP acts as a biological sensor for iron—when iron is abundant, IRP is a functional aconitase; when iron is scarce, the essential iron–sulfur cluster in the active site is destroyed, and IRP becomes an RNA-binding protein. Therefore, when iron is scarce, active IRP binds to ferritin message and prevents ferritin synthesis, whereas iron abundance releases the IRP from the message and promotes ferritin synthesis. Insect ferritin cDNAs so far investigated contain IREs, and two cDNAs encoding *Drosophila melanogaster* IRPs have been reported (5).

We have isolated a cDNA encoding a heavy-chain homologue (HCH) of a *D. melanogaster* ferritin subunit (FER1) (4). Using that cDNA to probe Northern blots of *D. melanogaster* mRNA revealed three bands of about 1.1, 1.3, and 1.5 kb. The intensity of the three bands varied with the developmental stage of the insect, especially under normal or iron-enriched dietary regimens. With the availability of a large number of clones from the Berkeley *Drosophila* Genome Project (BDGP), we have been able to show that the three bands seen on Northern blots actually represent at least four mRNAs, two of which contain IREs and two of which lack IREs. All of these clones encode the HCH reported earlier (4). The IRE is contained in a region of the gene that appears to be an intron, and alternative splicing of this intron results in mRNAs either containing or lacking an IRE. Significantly, iron enrichment shifts the population of mRNAs toward those lacking the IRE. We speculate that a nuclear form of IRP may control alternative splicing of the ferritin pre-mRNA.

As we were preparing this work for publication, a paper appeared with similar conclusions about the diversity of HCH messages in *D. melanogaster* resulting from alternative splicing (6).

MATERIALS AND METHODS

***Drosophila* Culture and Treatment.** The wild-type *D. melanogaster* Canton S strain was used throughout this study and was maintained on a standard cornmeal/agar medium at 22°C. For rearing insects on iron-rich diet, young larvae were transferred to a medium containing 5 mM $FeCl_3$. Third-instar larvae, pupae, and adults were collected from mass cultures on control and iron-enriched diets.

Isolation of RNA and Northern Blot Analysis. Total RNA was isolated from about 100 mg of control or iron-fed insects and from dissected larval guts and fat bodies by using a powdered glass affinity matrix (RNaid matrix, Bio 101) according to Noriega and Wells (7). RNA samples (30 μg) were electrophoresed on 1.2% agarose/formaldehyde gels (8) and transferred to GeneScreen Plus nylon membranes (DuPont/NEN). Blots were probed at high stringency [50% formamide, 5× SSPE (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA), 5× Denhardt's solution, 1% SDS] with several ferritin cDNA probes (Fig. 2). Probe A was a 1,263-bp *Eco*RI–*Xho*I fragment from clone LD03437 (see Table 1); probe B (166 bp)

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Abbreviations: HCH, heavy chain homologue; FER1, *Drosophila melanogaster* ferritin subunit 1; UTR, untranslated region; IRE, iron responsive element; IRP, iron regulatory protein; BDGP, Berkeley *Drosophila* Genome Project.

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Table 1. *D. melanogaster* FER1 HCH cDNAs

cDNA library	cDNA clones with IRE	cDNA clones without IRE
BDGP 0–24 h embryos	LD03479*, LD09059, LD04770*, LD05211, LD30974, LD11229*, LD25232, LD03437*, LD29529, LD02635*, LD04688*, LD14202, LD04328*, LD25105	LD21847, LD16801, LD10086*, LD16381, LD30494, LD11678*
BDGP larvae and early pupae	LP01702, LP04816, LP01150	LP01502
BDGP adult heads	GH05859, GH09985, GH07013†	GH14731, GH03196, GH05363, GH07449, GH07308, GH09912, GH07461, GH07052, GH05291‡, GH12784, GH07945
BDGP ovaries	GM02826, GM06304, GM03006	GM14556‡
λZap Canton S third-instar larvae	Df 4.3†	Df 2.2
5′ RACE Oregon R adults	R 500	

*Both ends of these cDNAs have been sequenced.
†Clones that are presumably with IRE since they end 11 and 4 bp, respectively, before the intron 3′ splice site at position −74.
‡cDNAs resulting from the use of an intron 3′ splice site at position −74.
RACE, rapid amplification of cDNA ends.

was generated by PCR using clone LD04770 as a template and the primers GGTACTGGAAAGCATTCG (5′) and GCACAAATGAGAATGAGG (3′); probe C (188 bp) was also generated by PCR with clone LD04770 as a template and primers CGTCTGTTCTTTCTTATCAACC (5′) and GAACTTGCTCGTCTAATTG (3′). The blots also were hybridized with an *Eco*RI–*Hin*dIII fragment of the rp49 gene as an internal loading control (9). All probes were gel-purified by using the Sephaglas BandPrep kit (Amersham Pharmacia), labeled with [α-^{32}P]dCTP by using the RadPrime DNA labeling system (GIBCO/BRL), and purified with the ProbeQuant G-50 micro columns (Amersham Pharmacia). Before hybridization (16–18 h at 42°C), the blots were incubated for 2–4 h in the above solution supplemented with 100 μg/ml salmon sperm DNA. After hybridization, the blots were washed in 2× SSPE/0.1% SDS twice at room temperature and once at 60°C for 20 min. The ferritin hybridization signals were normalized to the rp49 signal by using a PhosphorImager (Molecular Dynamics). The relative levels of induction of the 1.5-kb message, present in all insect groups and stages, were calculated by dividing the normalized message levels in the iron-fed insects to those in the control insects. To determine the relative contribution of different messages to the 1.3-kb band on the Northern blots, we calculated the ratios of the normalized 1.3-kb signals to the normalized 1.5-kb signals, both obtained with message-specific probes.

Database Searches and DNA Sequence Analysis. The collection of expressed sequence tags generated by BDGP, available at http://www.fruitfly.org, was searched for ferritin cDNA clones by using text strings and with the FER1 HCH subunit cDNA sequence with GenBank accession no. U91524 (4). Eleven of the identified cDNA clones were obtained, and the inserts were partially sequenced from both ends. To check for possible length polymorphism, the unsequenced internal segments of the cDNAs were amplified by using PCR with the primers GGAGATTTCAAGTGCTCCC (5′) and CTTGTCGAACAGGAACTCGCC (3′). A previously obtained FER1 HCH cDNA clone (4), Df2.2, was completely sequenced. DNA sequencing was performed by the automated DNA sequencing facility of the Arizona Research Laboratories Division of Biotechnology, University of Arizona. DNA sequences were analyzed by using the GCG sequence analysis software package (Genetics Computer Group, Madison, WI).

RESULTS AND DISCUSSION

Fig. 1 shows the result of hybridizing Northern blots of *D. melanogaster* RNA with a probe (probe A) that contains the entire coding region of the *D. melanogaster* ferritin HCH. The three bands represent at least four mRNAs, as reported by Lind *et al.* (6). We deduced the structures of these mRNAs by examination of cDNAs we had previously obtained by screening a library (4), as well as a number of clones identified from expressed sequence tags from BDGP, and the deduced structures are shown in Fig. 2. Table 1 lists the various cDNAs used in this analysis. Our results confirm those reported by Lind *et al.* (6) that these four mRNAs result from alternative splicing, and that the IRE is contained in an intron that may be removed in some of the messages. Because this intron can be removed by using three different 3′ splice sites (at positions −21, −73, and −113 relative to the ORF), the resulting mRNAs lacking IRE differ from the message with an unspliced intron by 224, 172, and 132 nt, respectively. Of these, only the messages resulting from the use of the most distal 3′ splice site (at position −21) are abundant, as found by both Northern blot and cDNA sequence analysis. The abundance of the other two alternatively spliced messages may increase under iron-loading conditions and/or in certain tissues. This is supported by the

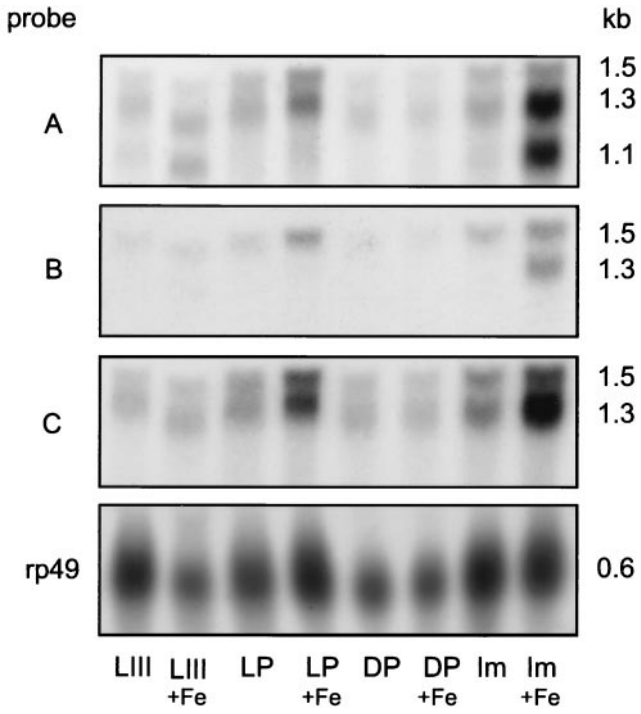

FIG. 1. Developmental expression and induction pattern of *D. melanogaster* ferritin HCH mRNAs. Autoradiograms of a Northern blot prepared with total RNAs and probed consecutively with three *Fer1 HCH*-specific probes (A, B, and C; see Fig. 2 for the size and location of the probes) and with rp49. The sizes of the massages detected on the blot are indicated on the right. LIII, third-instar larvae; LP, light pupae; DP, dark pupae; Im, imagoes; +Fe, insects raised on $FeCl_3$-supplemented diet.

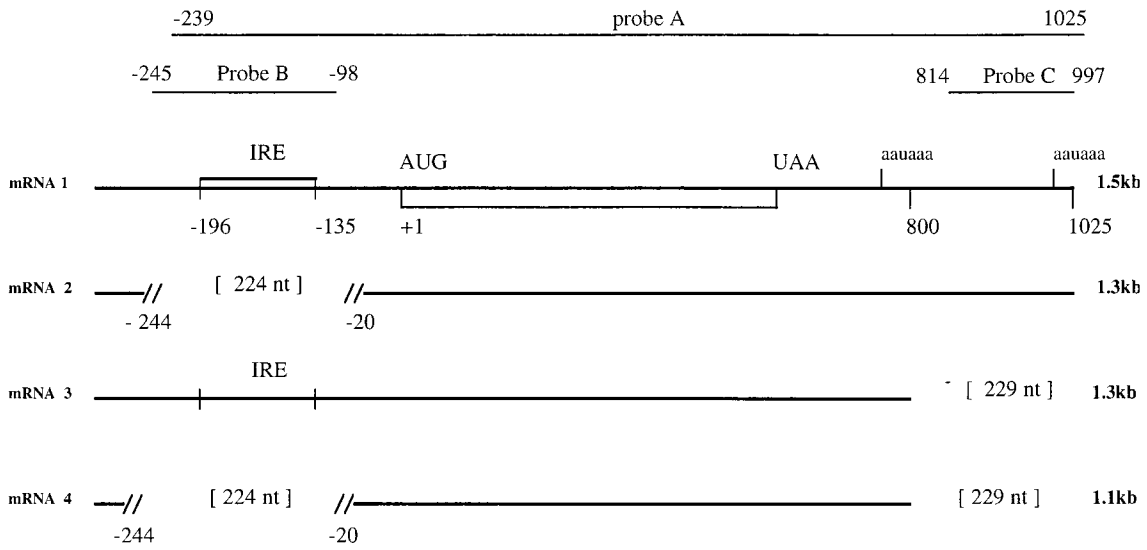

FIG. 2. Schematic representation of *D. melanogaster* FER1 HCH mRNAs. Four mRNAs are represented as horizontal lines. The IRE, the ORF, and the two putative polyadenylation signals are indicated on the longest mRNA (mRNA 1). The fragments used to probe Northern blots are shown as thin lines above the diagram. The numbers refer to coordinates on mRNA 1. The numbers in square brackets indicate the length and the location of the missing fragments in mRNAs 2, 3, and 4, as compared with mRNA 1. Messages 2 and 4, respectively, depict the most abundant species of two groups, each including three distinct messages without an IRE (see text for more details).

observation that on Northern blots, the bands resulting from these messages (1.3 kb and 1.1 kb) are more diffuse compared with the sharp 1.5-kb band that corresponds to the message with an unspliced intron. Thus, mRNA 2 and mRNA 4, depicted in Fig. 2 with the 224-nt intron removed, represent two classes of mRNAs lacking an IRE, each including three distinct alternatively spliced messages. Because the absence of the IRE is the most important feature of these messages, their microheterogeneity will not be discussed further. Also, presence of two different poly(A) signals at the 3′ end of the pre-mRNA gives rise to two types of mRNA with different lengths of 3′ UTR. This was also shown by Lind *et al.* (6), and is similar to the situation in human brain and liver, where ferritin heavy-chain mRNAs of different 3′ lengths have been seen (10). PCR amplification of the unsequenced central portions of nine FER1 HCH clones from the expressed sequence tag collection did not reveal additional length polymorphism.

Fig. 1 also shows that the abundance of the mRNAs differs with the life stage, and that messages become much more abundant when the animals are exposed to iron in the diet. Most significantly, the distribution of the different mRNAs is dramatically shifted by iron feeding. The Northern blots were hybridized with additional probes that allowed us to identify which messages were changing in abundance. The probes are shown in Fig. 2. Probe B identified those messages that contain an IRE, whereas probe C identified those with the longer 3′ UTR. Comparison of the Northern blots on Fig. 1 obtained with probes A, B, and C shows that, although all four messages are constitutively expressed, iron feeding strongly increases ferritin message abundance, and the messages lacking an IRE are very much increased.

Both sequence and Northern analyses suggested that the 1.5-kb band on the Northern blots corresponds to a single type of message (mRNA 1, Fig. 2) that is detected with all of the probes used (Fig. 1). To determine how the relative abundance of this message changes under iron-loading conditions and if all of the probes used faithfully reported these changes, we calculated the ratios of the normalized 1.5-kb hybridization signals in iron-loaded insects to those in control insects. The ratios, calculated for the Northern blots hybridized with probes A, B, and C (Table 2), clearly indicate that the 1.5-kb mRNA level is increased in third-instar larvae, early pupae, and flies raised on iron-supplemented diet. This increase is most prominent in early pupae and in adults, where the relative abundance of the message doubles. No increase of the message level is seen in the late pupae. The ratios obtained with different probes were very similar, suggesting that these probes correctly report changes in the abundance of the 1.5-kb mRNA. These results indicated that the levels of this message can be successfully used as a basis to calculate the relative abundance of the 1.3-kb band on the Northern blots to estimate the relative contribution of mRNA 2 and mRNA 3 to this band by using message-specific probes.

The ratios of the normalized 1.3-kb to 1.5-kb signals are presented in Table 3. These ratios indicate that in the pupal stages, mRNAs producing 1.5- and 1.3-kb bands on the Northern blots are increased coordinately. Thus, their levels increase two-fold in iron-overloaded light pupae, whereas no increase is seen in iron-loaded dark pupae (Table 2). In addition, the lack of 1.3-kb band signal detected with probe B indicates that this band is formed entirely by mRNA 2. In contrast, the levels of the 1.3-kb mRNAs increase significantly relative to those of the 1.5-kb mRNA in both larvae and imagoes, as revealed with probe A. In addition, the results obtained with probe B and C indicate that the contribution of mRNA 2 and mRNA 3 to this increase is different in larvae and imagoes. In larvae, the relative increase of the 1.3-kb band results almost entirely from a greater abundance of mRNA 2 (without IRE). In contrast, in imagoes both mRNA 2 and mRNA 3 contribute to the

Table 2. Relative induction levels of the 1.5-kb FER1 HCH message

Ratio	Probe		
	A	B	C
LIII + Fe/LIII	1.3	1.3	1.2
LIII + Fe/LIII (gut)	4.0	4.5	4.0
LIII + Fe/LIII (fat body)	0.8	0.8	0.8
LP + Fe/LP	1.8	2.0	2.0
DP + Fe/DP	1.1	1.1	1.0
Im + Fe/Im	1.7	2.0	1.8

The length and location of probes A, B, and C are shown in Fig. 2. The 1.5-kb FER1 HCH mRNA hybridization signals were normalized to the rp49 signals, and the ratios of the levels in iron-loaded insects to those in control insects were calculated. Abbreviations are as in Fig. 1.

Table 3. Relative levels of the 1.3-kb FER1 HCH mRNAs

Stages	Probe A	Probe B	Probe C
LIII	1.1	0.4	0.78
LIII (gut)	1.8	0.55	1.38
LIII (fat body)	1.04	0.27	0.8
LIII + Fe	1.84	0.5	1.4
LIII + Fe (gut)	2.1	1.08	1.13
LIII + Fe (fat body)	1.26	0.43	0.8
LP	1.1	—	1.1
LP + Fe	1.1	—	1.04
DP	1.0	—	1.0
DP + Fe	1.0	—	1.0
Im	1.1	0.29	0.9
Im + Fe	1.8	0.76	1.2

The length and location of probes A, B, and C are shown on Fig. 2. The FER1 HCH mRNA hybridization signals were normalized to the rp49 signals, and the ratios of the 1.3-kb mRNA levels to the 1.5-kb mRNA levels were calculated. Abbreviations are as on Fig. 1.

increase in the 1.3-kb band level: mRNA 3 (with IRE and short 3′ UTR) increases 100%, and mRNA 2 increases 30%. Such different effects of iron feeding on the ferritin messages in pupal vs. larval and imago stages is expected because the pupae are not exposed directly to extra ingested iron. In early pupae, when intensive histolysis and tissue remodeling takes place, increased message levels, and presumably, increased ferritin synthesis, may be needed to sequester iron and thus prevent possible oxidative damage. Similarly, up-regulation of ferritin at both transcriptional and translational level has also been implicated in the protection of mammalian cells from oxidative damage (11).

A Northern blot of RNAs isolated from third-instar larval guts and fat bodies was hybridized with probes A (Fig. 3), B, and C (data not shown). These results indicated that the quantity of all four FER1 HCH messages is regulated in an organ-specific manner. Levels of the 1.5- and 1.3-kb messages in these organs are quite different under both normal and iron-supplemented dietary regimens. Thus, the normal levels in the gut compared with those in the fat body are 3-fold higher for the 1.5-kb message and 5-fold higher for the 1.3-kb messages. These results indicate that the gut is a site where large quantities of ferritin are synthesized for sequestering the absorbed iron from the diet. This observation also is in agreement with the proposed function of insect ferritins in iron transport between tissues (2). In the gut of larvae raised on

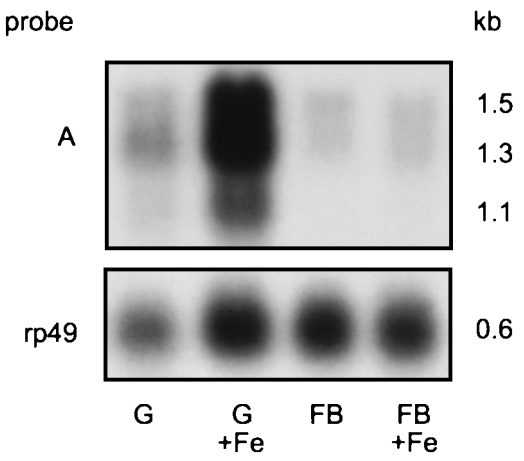

FIG. 3. Organ-specific expression of the FER1 HCH mRNAs. Northern blot of total RNAs isolated from third-instar larval organs, hybridized with *Fer1 HCH* probe A (see Fig. 2) and rp49. G, gut; FB, fat body; +Fe, organs from larvae raised on $FeCl_3$-supplemented diet.

iron-supplemented diet, the 1.5-kb FER1 HCH message is increased 4-fold, whereas in the fat body it is slightly decreased (Table 2). In addition, analysis of the hybridization signals obtained with message-specific probes (Table 3) indicates that the greater level of the 1.3-kb band in the larval gut under normal conditions is a result of the accumulation of mRNA 2 (without IRE). In contrast, in the guts of larvae fed an iron-supplemented diet, there is a 2-fold increase in the relative level of mRNA 3 (with IRE and with short 3′ UTR). At the same time, the mRNA 2 level closely follows the increase in the 1.5-kb mRNA level. These changes in the mRNA 2 and mRNA 3 in the gut of iron-overloaded larvae result in the increase (≈20%) in the intensity of the 1.3-kb band (relative to the 1.5-kb message) detected with probe A. Changes in the 1.3-kb mRNA levels compared with the 1.5-kb mRNA levels in the fat body of iron-fed larvae are less prominent and consist only of a 30% increase in the mRNA 3 level. Taken together, these results indicate that the larval gut is the major site where four ferritin HCH messages are present at high levels and these levels are regulated in a complex manner under conditions of iron overload.

Under normal conditions, mRNA 2 (without an IRE) is present at a high level in the larval gut and it increases proportionally with the unspliced message (mRNA 1) under high-iron conditions. This observation suggests that messages avoiding translational control via IRPs are essential for the rapid response of the gut to dietary iron. Such rapid massive synthesis of ferritin is probably needed on one hand for capturing iron when it is scarce in the diet, and on the other hand for sequestering iron when it is overabundant and can cause oxidative damage. In addition, production of messages with short 3′ UTR is dramatically increased under high-iron conditions in the larval gut. Similarly, these messages increase in the iron-fed imagoes, again most probably in the gut. There is evidence that differences in the length of the 3′ UTR could affect the stability, translational efficiency, or localization of various mRNAs (12). In addition, a portion of the 3′ UTR was shown to be involved in the repression of *in vitro* translation of the bullfrog ferritin M subunit mRNA (13). If ferritin mRNA 3 and mRNA 4 indeed have higher translational efficiency, they would also result in a rapid synthesis of ferritin subunits, providing a means to deal effectively with the extra dietary iron. Such an interpretation is supported by the results presented here, showing that FER1 HCH mRNA 4 (without IRE and with short 3′ UTR) is found at high levels only under high-iron conditions and probably only in the gut of both larvae and imagoes.

High-iron conditions result in an increase of all FER1 HCH message types. In addition, the messages without IRE (mRNA 2), with shorter 3′ UTR (mRNA 3), and both without IRE and with shorter 3′ UTR (mRNA 4) become more abundant relative to the full-length message (mRNA 1). These results suggest that under conditions of iron overload, when rapid synthesis of ferritin subunits is needed, two regulatory mechanisms come into play: (*i*) an overall increase in the FER1 HCH message levels and (*ii*) a shift of the balance toward shorter FER1 HCH messages achieved at the level of mRNA maturation.

What mechanisms are operating to alter the abundance and ratios of the various messages after iron feeding? Changes in the amount of message could result either from altered rates of transcription or degradation. Regulation by iron of ferritin synthesis at the transcriptional level is well documented in vertebrates (14) and in plants (15). Increased transcription of ferritin genes by iron supplementation has been demonstrated in mosquito cells in culture (D. Pham, personal communication). The increases seen in ferritin message abundance after iron feeding might thus result from increased transcription. However, they could also result from increased stability of messages, especially mRNAs 2, 3, and 4. Indeed, if translation

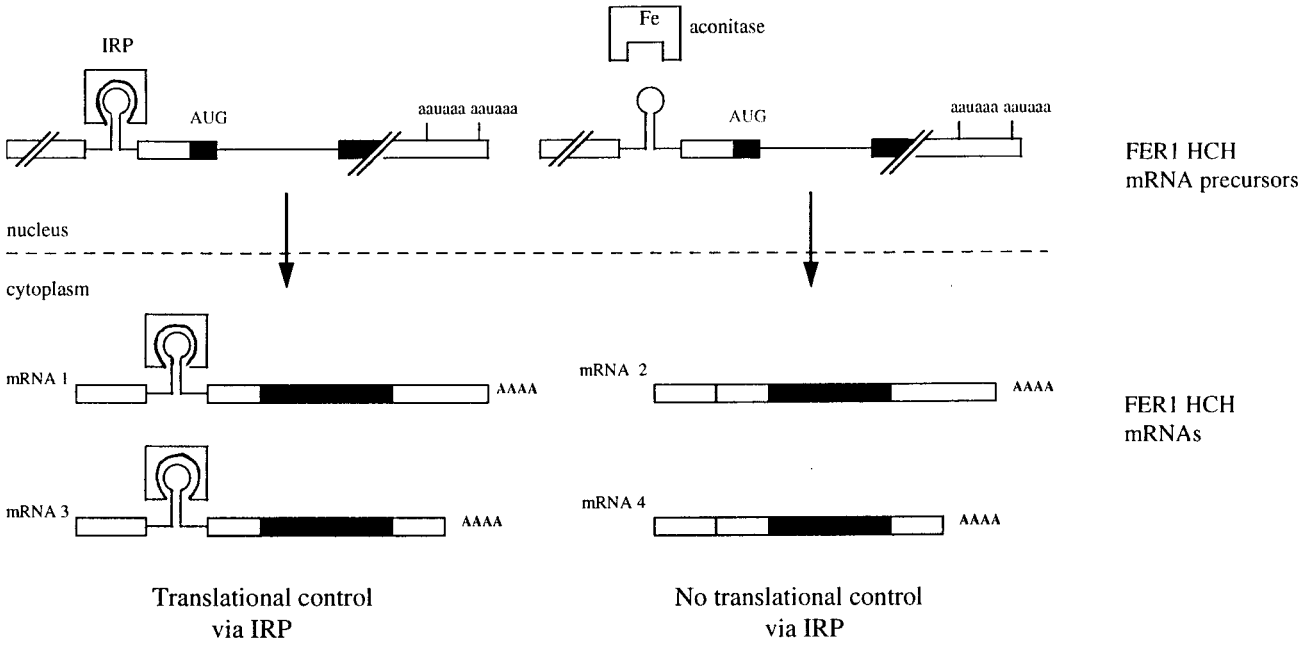

FIG. 4. Proposed model for alternative splicing of the FER1 HCH mRNA precursors mediated by IRP. Iron overabundance directs the conversion of IRP to a form with no RNA-binding activity (aconitase). This would leave the intron containing the IRE accessible for excision. Both spliced and unspliced messages would be produced constitutively, but high iron availability would shift the equilibrium toward the spliced messages. In addition, the use of two alternative polyadenylation sites would result in four mature mRNAs.

of these messages is enhanced, their stability may be increased. If absence of an IRE and a shorter 3′ end results in more efficient translation, then messages with these features would be favored. However, this explanation cannot account for the fact that mRNA 4, which is nearly undetectable in normal animals, becomes the most abundant form in iron-overloaded animals because it should be efficiently translated under both conditions.

A second attractive hypothesis is that the message distribution seen in iron-fed animals is the result of an effect of iron on the splicing process. Based on what is known about the translational control of protein synthesis by iron, one can speculate that the binding of a trans-acting factor to the IRE contained in the ferritin pre-mRNA might prevent splicing of the IRE-containing intron. Although this trans-acting factor might be a previously unknown protein, the simplest explanation would be that it is IRP-like or IRP itself. We propose a mechanism of this alternative splicing that involves IRPs (Fig. 4). It has recently been demonstrated that IRP is not present in the nucleus of mammalian cells (16), but we have no data for *Drosophila* cells. Recently, the primary sequences of two IRPs from *Drosophila*, very similar to each other and to the mammalian IRP 1, were reported (5). Their apparent function is to regulate the translation of various mRNAs, possibly including ferritin HCH mRNAs, by reversibly binding to IREs, but it is not clear what is the functional significance of the presence of two IRPs. In addition, despite the fact that *in vitro* binding of recombinant human IRP to the IRE of the FER1 HCH mRNA recently was shown (6), translational regulation of ferritin synthesis via IRPs has yet to be demonstrated in *Drosophila*. It is possible that *Drosophila* IRPs might have diverse and even different regulatory functions compared with their mammalian counterparts. Based on the results presented here, we propose that one (or both) *Drosophila* IRP 1 homologues may migrate to the nucleus where, by binding to the IRE of FER1 HCH mRNA precursors, it would serve as a regulator of their alternative splicing. In normal conditions, a fraction of the IRP/aconitase in its active IRE-binding form would migrate to the nucleus, where it would bind to the IRE of a fraction of the FER1 HCH mRNA precursor and prevent the excision of the intron that contains the IRE. As a result, both unspliced messages with IREs (eventually subject to translational control by cytoplasmic IRPs) and spliced messages (lacking an IRE) would be produced. Under conditions of iron overabundance, most of the cytoplasmic IRP loses its IRE-binding activity and becomes functional aconitase. In addition, the transcription of the *Fer1 HCH* gene may be up-regulated several-fold. The nuclear fraction of IRP under these conditions would not be sufficient to saturate the newly synthesized ferritin mRNA precursors. As a result, the intron containing the IRE would become accessible for excision, resulting in the accumulation of more messages without IREs. These messages would escape translational control via IRPs. A similar dual function as both a splicing and a translation regulator was demonstrated for the *Drosophila* Sex-lethal protein (17). This mechanism, like the differential message stability mechanism, does not account for the large increase in messages with short 3′ ends.

While the mechanism by which the altered distribution of messages is achieved remains to be explored experimentally, the changes following iron feeding suggest that iron has a dramatic effect on ferritin synthesis under conditions of iron overload.

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