dophila was determined to be 61 fs (*11*). The carotenoid in this complex, rhodopin glucoside, like lycopene has 11 conjugated double bonds. If we take the time scale for the S_2 to S_x transition from this report, then at least some of this energy transfer must involve S_x. Indeed, if S_2 was the only state from which energy transfer could occur, then the efficiency would be expected to be much lower than the measured value of this LH2 complex, which is 50%. This report illustrates again the remarkable subtlety of the photophysical behavior of carotenoids. The exact electronic designation of S_x and its involvement in the molecular mechanisms of carotenoid-to-chlorophyll singlet-singlet energy transfer remain to be determined.

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Rates of Behavior and Aging Specified by Mitochondrial Function During Development

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To explore the role of mitochondrial activity in the aging process, we have lowered the activity of the electron transport chain and adenosine 5′-triphosphate (ATP) synthase with RNA interference (RNAi) in *Caenorhabditis elegans*. These perturbations reduced body size and behavioral rates and extended adult life-span. Restoring messenger RNA to near-normal levels during adulthood did not elevate ATP levels and did not correct any of these phenotypes. Conversely, inhibiting respiratory-chain components during adulthood only did not reset behavioral rates and did not affect life-span. Thus, the developing animal appears to contain a regulatory system that monitors mitochondrial activity early in life and, in response, establishes rates of respiration, behavior, and aging that persist during adulthood.

During a systematic screen of a *C. elegans* chromosome I RNAi library (*1*, *2*), we found that animals grown on bacteria expressing double-stranded RNA (dsRNA) encoding a component of the mitochondrial ATP synthase (*atp-3*) lived much longer than normal (*3*) (Fig. 1A, table S1). RNAi of three genes encoding respiratory-chain components also extended life-span: *nuo-2*, which encodes a component of complex I (NADH/ubiquinone oxidoreductase); *cyc-1*, which encodes a component of complex III (cytochrome c reductase); and *cco-1*, which encodes a component of complex IV (cytochrome c oxidase) (Fig. 1A, table S1) (*3*). Treatment of wild-type animals with antimycin A, which inhibits complex III (*4*), increased life-span as well (*3*) (Fig. 1D, table S1).

RNAi of respiratory-chain components also affected growth and behavior. The animals were smaller than normal. They were well proportioned (fig. S1, table S2) and did not have an obvious decrease in cell number. We counted cells in two postembryonic lineages, the vulval and epidermal seam cell lineages (*5*), and found that cell number was normal (*3*). Thus, in these tissues (and likely in others as well), a metabolite whose level is regulated by mitochondrial respiration acts as a signal to control cell size. Small body size itself is unlikely to extend life-span, because mutants defective in *daf-4*, which encodes a transforming growth factor–β type II receptor (*6*), are small but not long-lived (*7*). Respiratory-chain RNAi also decreased the rate of growth to adulthood, as well as the rates of pharyngeal pumping (eating) and defecation (table S2). In addition, the animals moved more slowly than normal (table S2).

Inhibition of a component of respiratory chain complex III, *isp-1*, in *C. elegans* has been shown to reduce oxygen consumption (*8*). Inhibiting respiratory-chain components and ATP synthase would also be predicted to reduce ATP levels. We found that ATP levels were reduced 60 to 80% in animals subjected to *cyc-1* (complex III) or *atp-3* (ATP synthase) RNAi and 40 to 60% in animals treated with *nuo-2* (complex I) or *cco-1* (complex IV) RNAi (*3*) (Fig. 2).[6] These findings, and the fact that all these proteins are known to function together in the processes of respiration and ATP production, suggest that reducing the rates of respira-

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tion and ATP production decreases growth rate and body size, slows behavioral rates, and increases life-span.

The behavioral and longevity phenotypes of our RNAi-treated animals resemble those of *isp-1* mutants, except that *isp-1* mutants are not small (*8*). This suggests that our RNAi conditions are more severe. Mutations in the gene *clk-1* also slow developmental rates and behaviors and extend life-span (*9*, *10*). *clk-1* mutants cannot produce ubiquinone and instead acquire it from the bacteria they eat (*11*, *12*). However, the cause of the *clk-1* phenotype is likely to be different from that of our RNAi-treated animals. ATP levels are normal or slightly elevated in *clk-1* mutants (*13*), and surprisingly, we find that the longevity of *clk-1* mutants (but not their reduced behavioral rates) depends on the presence of the somatic gonad (fig. S2, table S2).

In *C. elegans*, inhibiting either insulin/insulin-like growth factor–1 (IGF-1) signaling or germ line proliferation extends life-span (*14*). Inhibiting insulin/IGF-1 signaling has been proposed to extend life-span by reducing mitochondrial activity (*8*, *15*). However, unlike the longevity produced by perturbing germ line or insulin/IGF-1 signaling, the longevity produced by these RNAi treatments were not dependent on the transcription factor *daf-16* (Fig. 1B, table S1). In addition, the life-span extensions of our RNAi-treated animals were increased much further by *daf-2* (insulin/IGF-1 receptor) mutations (Fig. 1C, table S1) and germ line ablation (fig. S2, table S1) (*3*). Finally, many long-lived insulin/IGF-1 mutants have normal growth and behavioral rates (*16*), and insulin/IGF-1 signaling mutants actually have much higher levels of ATP than normal (Fig. 2C) (*13*). Thus, these different perturbations appear to affect different life-span–regulatory pathways.

We next asked whether the rate of respiration acted in an ongoing manner throughout the life of the animal to affect behavioral rates and life-span or whether it was particularly important during a specific stage of life. First, we reduced respiratory-chain mRNA levels during development and then attempted to restore them during adulthood. To do this, we inhibited the RNAi machinery during adulthood by subjecting young adults treated with respiratory-chain RNAi from the time of hatching to *Dicer* (*dcr-1*) RNAi. The *dcr-1* gene is required for RNAi (*17*, *18*), and *dcr-1* RNAi reduces RNAi activity (*19*). Shifting

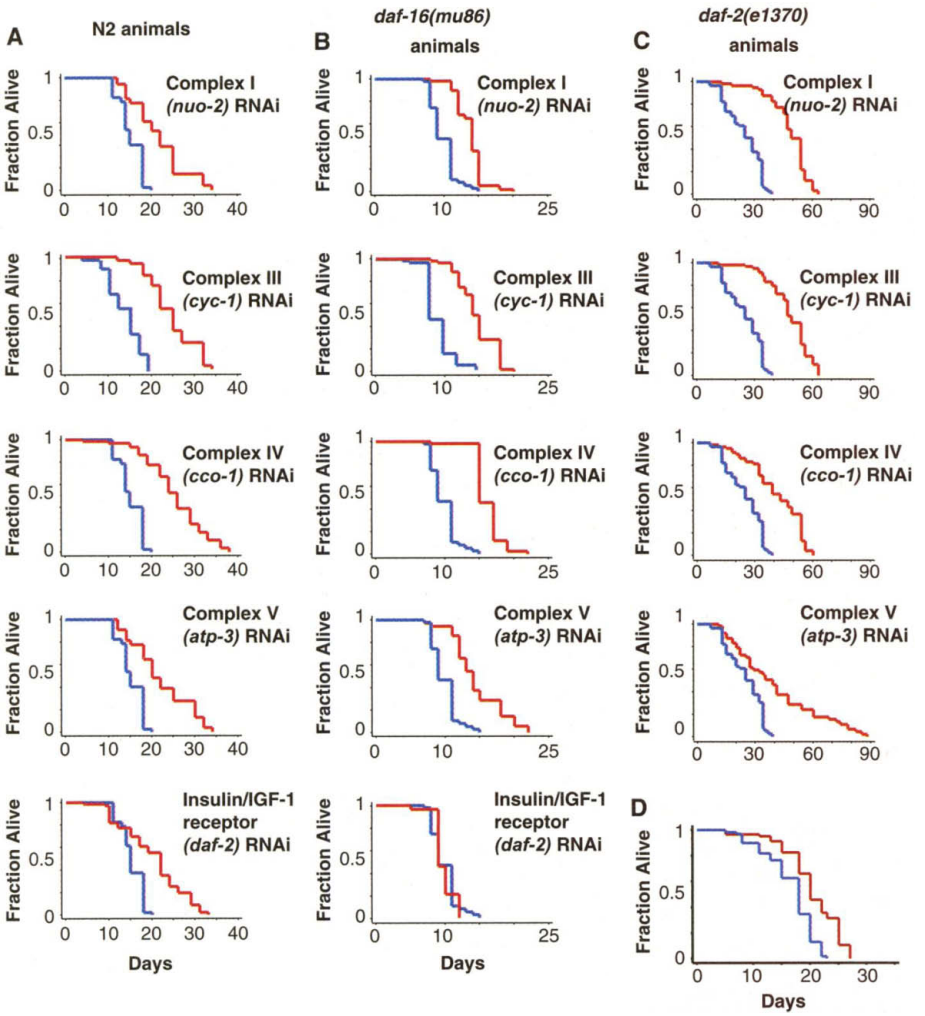

Fig. 1. Adult life-spans of (**A**) wild type (N2), (**B**) *daf-16*(*mu86*), and (**C**) *daf-2*(*e1370*) animals grown on bacteria expressing dsRNA of respiratory genes from the time of hatching. Blue lines, life-spans of animals grown on control bacteria containing the RNAi vector alone; red lines, life-spans of animals grown on bacteria expressing the indicated dsRNA. (**D**) Animals treated with antimycin A (red line), an inhibitor of respiratory-chain complex III, live longer than control animals (blue line). Statistical data can be found in table S1.

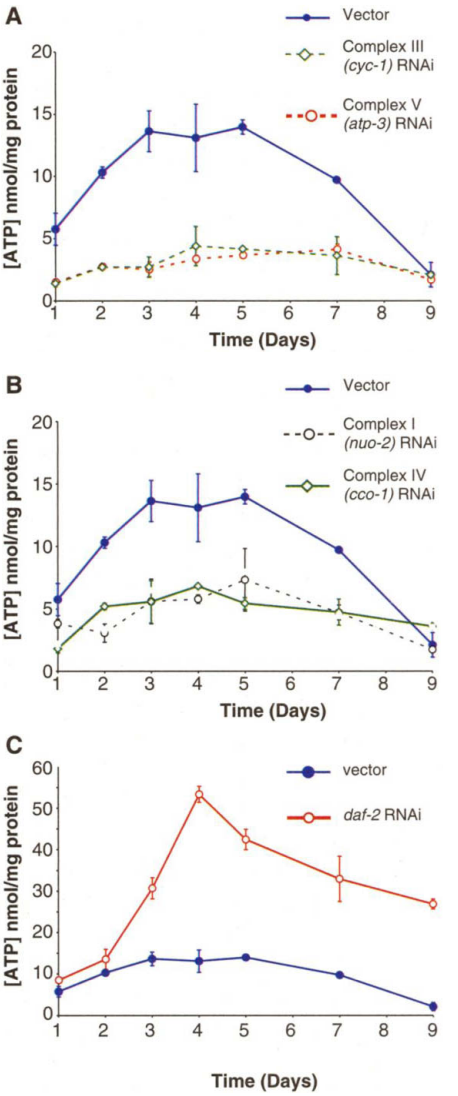

Fig. 2. RNAi of respiratory genes reduces ATP levels. Animals were exposed to dsRNA from the time of hatching. Solid blue lines represent the ATP levels of animals grown on control bacteria containing the RNAi vector alone. (**A**) Animals grown on bacteria expressing complex III (*cyc-1*) (green line) or complex V (*atp-3*) (red line) dsRNA. (**B**) Animals grown on bacteria expressing complex I (*nuo-2*) (black line) or complex IV (*cco-1*) (green line) dsRNA. (**C**) Animals grown on bacteria expressing *daf-2* dsRNA (red line). Note that the scales of (A) and (B) are different from that of (C).

Fig. 3. Reduction of *cco-1* (complex IV) expression during development is sufficient to lower the rate of metabolism during adulthood and increase life-span. To reduce *cco-1* expression during development, we grew animals on bacteria expressing *cco-1* dsRNA from hatching until the first day of adulthood. To increase *cco-1* expression in adulthood, we shifted these young adults to bacteria expressing *dcr-1* dsRNA. (**A**) Concurrent measurement of *cco-1* and *dcr-1* mRNA levels by reverse transcriptase–polymerase chain reaction (RT-PCR) during day 1 until day 3 of adulthood. As *dcr-1* mRNA levels fall, *cco-1* mRNA levels rise. Shown are RT-PCR products from serial dilutions of total RNA isolated from animals grown on the RNAi vector, *cco-1* dsRNA-expressing bacteria during development and then shifted to *dcr-1* dsRNA bacteria, or *cco-1* dsRNA-expressing bacteria during development and adulthood. (**B**) Concurrent ATP assays from the animals in (A). Increasing *cco-1* mRNA levels during adulthood does not increase ATP levels. Black line, ATP levels of control animals grown on bacteria containing the RNAi vector; red line, ATP levels of animals grown on bacteria expressing *cco-1* dsRNA during development and then shifted to *dcr-1* RNAi bacteria during day 1 of adulthood. Blue line, ATP levels of animals grown continuously on bacteria expressing *cco-1* dsRNA. (**C**) Red line, life-spans of animals grown on bacteria expressing *cco-1* dsRNA during larval development and then shifted on day 1 of adulthood to bacteria expressing *dcr-1* dsRNA for the remainder of their life. Blue line, life-span of animals grown on bacteria expressing *cco-1* dsRNA during development and adulthood. Black line, life-span of animals grown on bacteria containing the RNAi vector only.

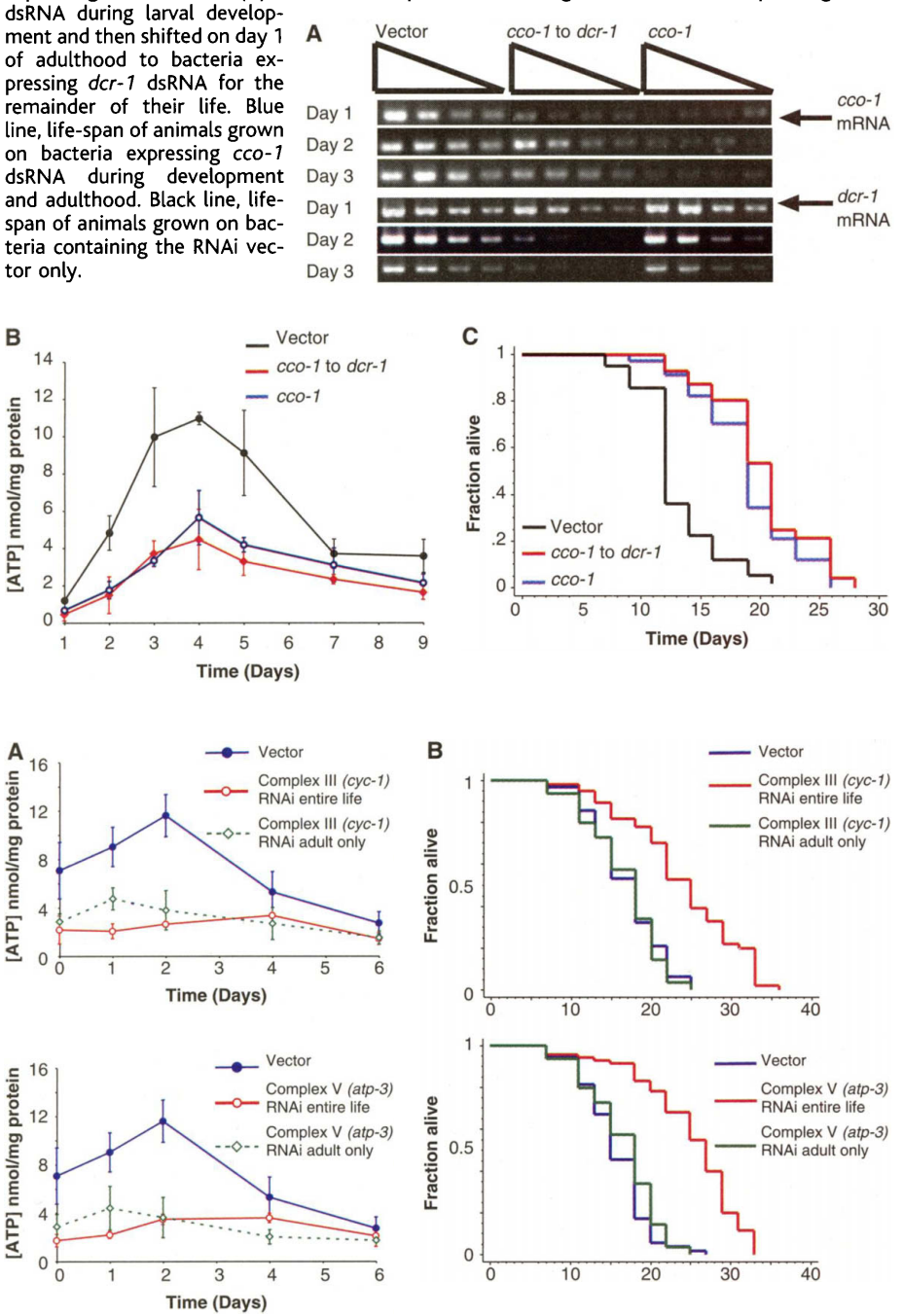

Fig. 4. Initiating RNAi of complex III (*cyc-1*) or complex V (*atp-3*) during adulthood lowers ATP levels (**A**) but does not extend life-span (**B**). Animals were grown until the first day of adulthood on normal bacteria and then shifted to bacteria expressing *cyc-1* (complex III) or *atp-3* (complex V) dsRNA. Control animals were shifted to bacteria containing the RNAi vector.

young adults grown on bacteria expressing *cco-1* (complex IV) dsRNA to bacteria expressing *dcr-1* dsRNA decreased *dcr-1* mRNA levels and greatly increased *cco-1* mRNA levels (Fig. 3A) (*3*). Unexpectedly, *dcr-1* RNAi treatment did not increase the level of ATP at all (Fig. 3B), nor did it correct any of the behavioral or longevity phenotypes caused by *cco-1* RNAi (Fig. 3C, table S1) (*3*). It was conceivable that these phenotypes were not corrected because the level of *cco-1* mRNA did not return fully to normal. However, our failure to see an increase in ATP levels commensurate with the large increase in mRNA we observed following *dcr-1(RNAi)* treatment suggests an alternative explanation, namely, that in these RNAi-reversed animals, something other than *cco-1* mRNA had become a limiting factor in ATP production.

Next, we inhibited respiratory-chain components only during adulthood by transferring animals as young adults onto bacteria expressing respiratory-chain dsRNA. Within 8 hours, their ATP levels were reduced to the same extent as in animals exposed to respiratory-chain dsRNA from hatching (Fig. 4A). Unexpectedly, the life-spans of these animals were not extended (Fig. 4B, table S1). It was conceivable that administering RNAi during adulthood did not decrease the level of ATP sufficiently to affect their physiology. However, this seems unlikely, because their mobility was greatly reduced as they aged and their pumping rates were decreased to the same extent as in animals exposed to RNAi from hatching (Table 1). Significantly, animals treated with RNAi from hatching displayed slow but regular pumping, whereas animals treated as young adults exhibited bursts of rapid pumping followed by pauses (see standard deviations in Table 1). This suggests the existence of a regulatory switch that establishes new periodic rates of pumping if respiratory-chain activity is inhibited from the time of hatching but not if it is inhibited during adulthood only.

We have shown that reducing the level of components of electron-chain complexes I, III, and IV or ATP synthase (complex V) from the time of hatching reduces body size and behavioral rates and increases life-span substantially. Surprisingly, in animals treated with respiratory-chain RNAi during development, restoring mRNA levels nearly to normal during adulthood did not increase the level of ATP. This suggests that reducing respiratory-chain activity during development permanently changes the system so that it continues to limit ATP production even if the original impediment is removed. In addition, although initiating respiratory-chain RNAi during adulthood lowered ATP levels just as much as when RNAi was initiated at hatching, the phenotypic effects were completely different. The animals were not capable of establishing new (uniform) pumping rates or of living

Table 1. Pumping rates of larvae and adults treated with respiratory chain RNAi.

RNAi treatment	Feeding rate (pumps per minute)*			
	Day 2	Day 3	Day 4	Day 5
Vector	118 ± 15 (0/8)	140 ± 21 (0/8)	145 ± 24 (0/8)	131 ± 49 (1/10)
Complex III (*cyc-1*) larval + adult	97 ± 21 (0/10)	83 ± 12 (0/10)	84 ± 12 (0/10)	75 ± 12 (0/10)
Complex III (*cyc-1*) adult only	79 ± 62 (5/12)	112 ± 80 (3/12)	80 ± 59 (4/12)	69 ± 45 (4/12)
Complex V (*atp-3*) larval and adult	98 ± 15 (0/10)	87 ± 10 (0/10)	80 ± 9 (0/10)	75 ± 8 (0/10)
Complex V (*atp-3*) adult only	88 ± 80 (6/14)	98 ± 64 (5/14)	83 ± 59 (4/14)	89 ± 54 (4/14)

*Mean ± SD. The number of pharyngeal pumps observed in an adult animal in 1 min at 25°C. Number in parentheses is the number of animals that produced fewer than 50 pumps per minute/total number of animals tested.

longer than normal. This lack of life-span extension was particularly unexpected because mitochondrial respiration is widely assumed to influence aging in an ongoing manner during adulthood through the generation of reactive oxygen species (*8*, *20*). Our findings bring this assumption into question.

Caloric restriction during adulthood extends life-span and has been proposed to act by decreasing the rate of respiration (*21*, *22*). However, our finding that life-span extension caused by respiratory-chain RNAi requires inhibition during development suggests that caloric restriction in animals, as in yeast (*23*), extends life-span in another way. The same holds for insulin/IGF-1 signaling, which functions exclusively during adulthood to influence *C. elegans* life-span (*19*).

In conclusion, we propose that *C. elegans* possesses a regulatory system that senses, interprets, and remembers the rate of mitochondrial respiration during development. Under normal conditions, this system establishes normal rates of growth, behavior, and aging. However, if the rate of respiration is low, this system reduces the animal's growth rate and body size, as well as its rates of behavior and aging. It is possible that the rate of respiration during development is sufficient to specify the rate at which the animal lives its entire life; alternatively, the adult animal may make reference to contemporaneous rates of respiration, which, in turn, are influenced by mitochondrial activity during development.

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Coordinated Nonvectorial Folding in a Newly Synthesized Multidomain Protein

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The low-density lipoprotein receptor (LDL-R) is a typical example of a multidomain protein, for which in vivo folding is assumed to occur vectorially from the amino terminus to the carboxyl terminus. Using a pulse-chase approach in intact cells, we found instead that newly synthesized LDL-R molecules folded by way of "collapsed" intermediates that contained non-native disulfide bonds between distant cysteines. The most amino-terminal domain acquired its native conformation late in folding instead of during synthesis. Thus, productive LDL-R folding in a cell is not vectorial but is mostly posttranslational, and involves transient long-range non-native disulfide bonds that are isomerized into native short-range cysteine pairs.

In eukaryotic cells, multidomain proteins are thought to fold their domains independently and sequentially (*1–3*). Examples of multidomain proteins have been found in the LDL-R family, which consists of a range of receptors that share structural elements (*4*, *5*). The LDL-R itself is a surface glycoprotein that mediates cellular uptake of LDL (*6*). It has been proposed that its ectodomain consists of three regions (Fig. 1A): the NH_2-terminally located ligand-binding region (composed of seven complement-like domains, each stabilized by three disulfide bonds and a calcium ion) (*7*, *8*), the epidermal growth factor (EGF) precursor-like region (*9*, *10*), and the abundantly O-glycosylated region. Structure determinations and in vitro folding studies of LDL-R fragments indicated a linear domain organization (Fig. 1A). This result suggests independent and sequential folding of the ligand-binding domains in the complete LDL-R (*11*, *12*).

Protein folding in the endoplasmic reticulum (ER) is tightly linked with disulfide bond formation in the newly synthesized protein (*13*, *14*). Whether non-native disulfide bonds are abundant or even essential in a folding pathway is still a matter of debate. Non-native bonds appear frequently in folding assays in vitro (*15–17*), but their occurrence in productive folding pathways in intact cells may be bypassed by the activity of protein

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