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Breakthroughs and Views

A novel hypothesis on the biochemical role of the *Drosophila* Yellow protein

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Abstract

In *Drosophila melanogaster*, the protein product of the *yellow* gene is necessary for normal pigmentation and male sexual behavior. Although one of the best characterized loci from a genetic standpoint, the function of the Yellow protein in the development of either phenotype is unknown. Here I propose that Yellow acts as a growth factor- or hormone-like molecule in the development of pigmentation and sexual behavior, and discuss the consistency of this theory with experimental observations in flies and humans.

That the protein product of the *yellow* (*y*) gene is necessary for normal *Drosophila melanogaster* pigmentation has been known since the first null mutant allele was discovered in 1911 by Edith Wallace [1]. Its necessity for normal male sexual behavior was discovered shortly thereafter [2]. Both results have been confirmed by numerous investigators [3,4].

The biochemical role of the Yellow protein in the development of either pigmentation or sexual behavior is currently unknown. At least two theories have been advanced concerning the biochemical mechanism by which Yellow is involved in pigmentation. Geyer et al. [5] proposed that Yellow is a structural protein which functions to crosslink a dopamine derivative, indole-5,6 quinone, during melanization, and there are a number of indirect lines of evidence consistent with this hypothesis. Alternatively, Wittkopp et al. [6] have proposed that Yellow is an enzyme in the melanin pathway downstream of dopa, based similarly on circumstantial evidence and also a significant sequence similarity between Yellow and a dopachrome conversion enzyme from a mosquito [7]. In the case of the former hypothesis, there is currently a lack of relevant experimental data directly bearing on its legitimacy. Regarding the latter, Han et al. [8] found that Yellow did not have the ability to act as a dopachrome conversion enzyme in an in vitro assay, providing evidence against the theory. (It is noteworthy that these authors did find the related Yellow-f and Yellow-f2 proteins to have such function, however [8].)

Neither of the above theories accounts for the necessity of Yellow for normal behavior. Here I advance a novel theory that in the development of behavior and pigmentation Yellow acts as either a hormone- or a growth factor-like molecule to bind to receptors and manipulate the growth, morphology or function of nearby cells.

Does Yellow resemble a typical hormone or growth factor?

Hormones and growth factors can have a variety of influences on the CNS during development, including functions related to secondary sex determination [9,10]. Does the Yellow protein resemble a typical polypeptide hormone or growth factor? The primary translation product of a future hormone often has 20–30 hydrophobic amino acids (aa) at the amino terminus, which act to direct the polypeptide through the endoplasmic reticulum (ER). In the ER, the signal peptide is cleaved and asparagine-linked glycosylation may occur. Consistent with this, Yellow has a 21 aa hydrophobic signal sequence and two putative asparagine glycosylation sites

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[5]. The predictions from the primary transcript sequence were later experimentally confirmed [11], demonstrating that Yellow carries a 21 aa signal peptide which is cleaved in the ER and is glycosylated. Furthermore, many polypeptide hormones are members of protein families, as Yellow is [12]. Biochemically, Yellow resembles a hormone or growth factor.

Is Yellow secreted from cells and does it bind to receptors on others?

There is evidence to suggest that Yellow is secreted from both cuticle [5,6,11,13] and neural cells [14,15]. Yellow is non-autonomous over short distances in the cuticle [13] and it visually appears to travel to other cells after being secreted from larval neuroblasts [14,15]. After secretion, Yellow may act as a hormone- or growth factor-like molecule, binding to receptors on other nearby cells in order to activate signal transduction pathways in those cells.

Which receptor(s) might Yellow bind to?

If Yellow is a secreted signal, it should bind to receptors on nearby cells. The identity of the receptor(s) is not known. However, what may be a significant clue comes from the mammalian pigmentation literature [16]. A hormone-activated cAMP pathway is known to mediate melanization via the melanocortin 1 receptor (MC1R). When either α-melanocyte stimulating hormone (α-MSH) or adrenocorticotropic hormone (ACTH) (which are both synthesized from the some prohormone precursor) binds to MC1R, intracellular cAMP levels are increased via adenylate cyclase. Although the many details connecting cAMP to melanogenesis are outside the scope of this paper (see [16]), protein kinase A and the CREB transcription factor are activated, and in turn other transcription factors are turned on, leading to up-regulation of genes relevant to melanin production.

There could be an analogous mechanism acting in invertebrates, in which Yellow function is equivalent to that of α-MSH and/or ACTH. This idea is consistent with the fact that interference with α-MSH or ACTH binding to MC1R and subsequent inactivation of the cAMP signal transduction pathway yields yellow colored mice [16]. If this hypothesis were correct, then the study of Yellow action can serve as a model for human skin variations and disorders, since it is known that interference with and variations in human α-MSH is associated with pigmentation levels [17–20].

What is the *Drosophila* equivalent of MC1R? Sequence comparisons [21,22] show that the proteins most closely resembling proteins in the melanocortin receptor family are a group of dopamine receptors. In *D. melanogaster* there are a number of these, DopR, DopR2, and DD2R, the last of which has at least 8 isoforms [23]. That dopamine, melanin, behavior, and Yellow may be related has previously been discussed at length [15,24]. Because it is common for a given hormone binding to a given receptor in different cell types to yield different responses, the notion that Yellow can change neural cell properties in one case and promote melanin production in another is reasonable.

Consistency of the theory with other experimental observations

This theory is consistent with experiments which show that *y* phenocopies for both pigmentation and behavior can be created using pharmacological inhibitors of tyrosine hydroxylase (TH), the rate-limiting enzyme in the metabolic pathway in which melanin and dopamine are synthesized, if we consider that the inhibitors acted downstream from *y* in a severe manner, whereas in *y* mutants it is possible that potential defects in the downstream part of the pathway are compensated for [24–29]. Our theory is also consistent with the idea that Yellow acts upstream of Yellow-f and Yellow-f2 in the melanin pathway [8]. Finally, our theory is consistent with the observation that *y* function appears to be necessary for normal learning behavior [30,31] and larval foraging behavior [32], each known to be dependent on the functioning of second-messenger systems.

If Yellow functions as a hormone or growth factor during metamorphosis, it may aid neurons in forming new motor connections and/or responding to novel sensory inputs in the adult. Observations suggest that male-specific FRU-mediated Yellow action in the male CNS begins in the 3rd instar [14,15]. This could explain how sex-specific male wing extension behavior [15,26] in response to pheromonal stimulation from females develops, if Yellow directs CNS control over muscles in males (but not females) which control wing extension and/or directs the chemosensory input which stimulates wing extension.

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References

[1] D.L. Lindsley, E.H. Grell, Genetic variations of *Drosophila melanogaster*, Carnegie Institution of Washington Publication No. 627 (1968).

[2] A.H. Sturtevant, Experiments on sex recognition and the problem of sexual selection in *Drosophila*, Journal of Animal Behaviour 5 (1915) 351–366.
[3] D.L. Lindsley, G.G. Zimm, The Genome of *Drosophila melanogaster*, Academic Press, San Diego, CA, 1992.
[4] M.D. Drapeau, A.G. Gibbs, W.S. Neckameyer, A.D. Long, Why do *yellow*^{1} males have poor mating success? Behaviour Genetics (in review).
[5] P.K. Geyer, C. Spana, V.G. Corces, On the molecular mechanism of *gypsy*-induced mutations at the *yellow* locus of *Drosophila melanogaster*, EMBO Journal 5 (1986) 2657–2662.
[6] P.J. Wittkopp, J.R. True, S.B. Carroll, Reciprocal functions of the *Drosophila* Yellow and Ebony proteins in the development and evolution of pigment patterns, Development 129 (2002) 1849–1858.
[7] J.K. Johnson, J. Li, B.M. Christensen, Cloning and characterization of a dopachrome conversion enzyme from the yellow fever mosquito, *Aedes aegypti*, Insect Biochemistry and Molecular Biology 31 (2001) 1125–1135.
[8] Q. Han, J. Fang, H. Ding, J.K. Johnson, B.M. Christensen, J. Li, Identification of the *Drosophila melanogaster yellow-f* and *yellow-f2* proteins as dopachrome-conversion enzymes, Biochemical Journal 368 (2002) 333–340.
[9] S.F. Gilbert, Developmental Biology, fifth ed., Sinauer Associates, Sunderland, MA, 1997.
[10] E.R. Kandel, J.H. Schwartz, T.M. Jessell, Principles of Neural Science, fourth ed., McGraw-Hill, New York, 2000.
[11] A. Kornezos, W. Chia, Apical secretion and association of the *Drosophila yellow* gene product with developing larval cuticular structures during embryogenesis, Molecular and General Genetics 235 (1992) 397–405.
[12] M.D. Drapeau, The family of Yellow-related *Drosophila melanogaster* proteins, Biochemical and Biophysical Research Communications 281 (2001) 611–613.
[13] A. Hannah, Non-autonomy of *yellow* in gynandromorphs of *Drosophila melanogaster*, Journal of Experimental Zoology 123 (1953) 523–560.
[14] A. Radovic, P.J. Wittkopp, A.D. Long, M.D. Drapeau, Immunohistochemical colocalization of Yellow and male-specific Fruitless in *Drosophila melanogaster* neuroblasts, Biochemical and Biophysical Research Communications 293 (2002) 1262–1264.
[15] M.D. Drapeau, A. Radovic, P.J. Wittkopp, A.D. Long, A gene necessary for normal male courtship, *yellow*, acts downstream of *fruitless* in the *Drosophila melanogaster* larval brain, Journal of Neurobiology 55 (2003) 53–72.
[16] R. Buscà, R. Ballotti, Cyclic AMP a key messenger in the regulation of skin pigmentation, Pigment Cell Research 13 (2000) 60–69.
[17] A.B. Lerner, J.S. McGuire, Effect of α and β melanocyte stimulating hormone on the skin colour of the man, Nature 189 (1961) 176–179.
[18] N. Levine, S.N. Sheftel, T. Eytan, R.T. Dorr, M.E. Hadley, J.C. Weinrach, G.A. Ertl, K. Toth, D.L. McGee, V.J. Hruby, Induction of skin tanning by subcutaneous administration of a potent synthetic melanotropin, Journal of the American Medical Association 226 (1991) 2730–2736.
[19] P. Valverde, E. Healy, I. Jackson, E. Rees, A. Thody, Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans, Nature Genetics 11 (1995) 328–330.
[20] H. Krude, H. Biebermann, W. Luck, R. Horn, G. Brabant, A. Gruters, Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans, Nature Genetics 19 (1998) 155–157.
[21] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, Journal of Molecular Biology 215 (1990) 403–410.
[22] FlyBase, The FlyBase database of the *Drosophila* genome projects and community literature, Available from http://flybase.bio.indiana.edu/, Nucleic Acids Research 31 (2003) 172–175.
[23] M.G. Hearn, Y. Ren, E.W. McBride, I. Reveillaud, M. Beinborn, A.S. Kopin, A *Drosophila* dopamine 2-like receptor: molecular characterization and identification of multiple alternatively spiced variants, PNAS USA 99 (2002) 14554–14559.
[24] B. Burnet, K. Connolly, Activity and sexual behaviour in *Drosophila melanogaster*, in: J.H.F. van Abeelen (Ed.), The Genetics of Behaviour, American Elsevier Publishing Company, New York, 1974, pp. 201–258.
[25] T.R.F. Wright, The genetics of biogenic amine metabolism, sclerotization, and melanization in *Drosophila melanogaster*, Advances in Genetics 24 (1987) 127–222.
[26] B. Burnet, K.J. Connolly, B. Harrison, Phenocopies of pigmentary and behavioral effects of the *yellow* mutant in *Drosophila* induced by α-dimethyltyrosine, Science 181 (1973) 1059–1060.
[27] R.D. Newcomb, D.M. Lambert, The sensitive period for *yellow* phenocopy induction in *Drosophila melanogaster*, Experientia 44 (1988) 618–621.
[28] R.D. Newcomb, The *yellow* condition in *Drosophila melanogaster*: a biological structuralist approach to the study of phenocopies, Rivista di Biologia 83 (1990) 381–396.
[29] W. Neckameyer, J. O'Donnell, Z. Huang, W. Stark, Dopamine and sensory tissue development in *Drosophila melanogaster*, Journal of Neurobiology 47 (2001) 280–294.
[30] R. Booker, A behavioral genetic analysis of learning in *Drosophila melanogaster*, Ph.D. dissertation, Princeton University, NJ, 1982.
[31] T. Tully, J.P. Gergen, Deletion mapping of the *Drosophila* memory mutant *amnesiac*, Journal of Neurogenetics 3 (1986) 33–47.
[32] K.A. Osborne, A. Robichon, E. Burgess, S. Butland, R.A. Shaw, A. Coulthard, H.S. Pereira, R.J. Greenspan, M.B. Sokolowski, Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*, Science 277 (1997) 834–836.