# All Members of the MHC Multigene Family Respond to Thyroid Hormone in a Highly Tissue-Specific Manner

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**In mammals different isoforms of myosin heavy chain are encoded by the members of a multigene family. The expression of each gene of this family is regulated in a tissue- and developmental stage–specific manner as well as by hormonal and various pathological stimuli. In this study the molecular basis of isoform switches induced in myosin heavy chain by thyroid hormone was investigated. The expression of the myosin heavy chain gene family was analyzed in seven different muscles of adult rats subjected to hypo- or hyperthyroidism with complementary DNA probes specific for six different myosin heavy chain genes. The results demonstrate that all six genes are responsive to thyroid hormone. More interestingly, the same myosin heavy chain gene can be regulated by thyroid hormone in highly different modes, even in opposite directions, depending on the tissue in which it is expressed. Furthermore, the skeletal embryonic and neonatal myosin heavy chain genes, so far considered specific to these two developmental stages, can be reinduced by hypothyroidism in specific adult muscles.**

THE MAXIMUM VELOCITY OF CONTRACTION in heart and skeletal muscle has been related to the specific activity of myosin adenosinetriphosphatase (*1*). Immunological, peptide mapping, and amino acid sequence analyses have demonstrated the existence of closely related but distinct isoforms of myosin heavy chain (MHC) (molecular weight, 200,000), a principal subunit of myosin (*2*). More recently, by the use of complementary DNA (cDNA) and genomic DNA clones, it was demonstrated that multiple genes, which appear to be clustered on the same chromosome (*3*), encode these MHC isoforms (*4*). Despite their similarity in primary structure, expression of different MHC isoforms is precisely regulated in a tissue- and developmental stage–specific manner (*4, 5*). In addition, innervation patterns (*6*), altered physiological stimuli (*7*), and various hormones (*8*) are known to cause MHC isoform switches.

The precise molecular basis of the MHC isoform switches, however, is not well understood. In rats (*9*) and rabbits (*10*), the cardiac ventricular α- and β-MHC transitions produced by changes in thyroid hormone level are regulated at the level of the respective messenger RNA (mRNA) availability, which in turn appears to be transcriptionally regulated. Furthermore, the α- and β-MHC genes are also expressed in extraventricular muscles (*9–11*).

To determine whether the same MHC gene will respond to thyroid hormone in the same manner when expressed in different tissues, we analyzed by S1 nuclease mapping the expressions of six distinct MHC genes in seven muscles that represent a broad range of fiber phenotypes in normal, hypothyroid, and hyperthyroid rats. The cDNA probes used correspond to the cardiac α- and β-MHC genes and to the skeletal embryonic, neonatal, fast IIA, and fast IIB MHC genes (*12*), which are all the MHC genes presently known to be expressed in rat cardiac and skeletal muscles (*4*), with the exception of an extraocular muscle-specific MHC gene (*13*). The results presented here demonstrate that each MHC gene analyzed displays an unexpected heterogeneity in its response to thyroid hormone when expressed in different muscles. Furthermore, we show that the embryonic and neonatal MHC genes can be reinduced in specific adult muscles by hypothyroidism.

The pattern of expression of the cardiac α- and β-MHC genes was analyzed by using as a probe the 3′ end Pst I fragment of pCMHC5, a cDNA clone specific for the β-MHC gene. With this probe, β-MHC mRNA was shown to yield a fully protected fragment 304 nucleotides (nt) long, while α-MHC mRNA produced a 180-nt partially protected fragment (*9*). As shown in Fig. 1A, there is little or no β-MHC mRNA in the ventricle and atrium of adult control euthyroid animals. Hypothyroidism, however, causes a dramatic induction of this gene in the ventricle, whereas in the atrium its induction is less pronounced. The β-MHC gene is also expressed in the soleus and diaphragm, in agreement with our previous observations that, in the rat, the type I (slow oxidative) skeletal MHC and the cardiac β-MHC are encoded by the same β-MHC gene (*9*). The level of expression of this gene, already high in normal soleus and diaphragm, does not seem to increase in hypothyroid animals. In contrast, hyperthyroidism clearly deinduces this gene in these skeletal muscles, although not to the same degree as in the atrium and ventricle. These results appear to indicate that physiological levels of thyroid hormone are already sufficient to suppress expression of this gene in the atrium and ventricle, but not in the soleus and diaphragm, where it remains expressed even in hyperthyroid animals. Thus, although expression of the β-MHC gene is down-regulated by thyroid hormone in all tissues analyzed, its basal level of expression and its responsiveness to the hormone vary among different muscles.

The α-MHC mRNA, identified by the partially protected 180-nt fragment (Fig. 1A), is present only in the atrium and ventricle, indicating that the α-MHC gene is expressed only in cardiac muscle (Fig. 1A). Expression of this gene does not change in the atrium in the hypo- or hyperthyroid state, whereas in the ventricle it is significantly deinduced by hypothyroidism. Thus expression of the α-MHC gene appears to be independent of thyroid hormone in the atrium but highly dependent in the ventricle. The same result was obtained with the α-MHC gene–specific cDNA probe pCMHC26 (*9*).

Figure 1B shows the expression of the type IIA (fast oxidative) MHC gene in five different muscles. The fully protected 360-nt fragment of clone pMHC40 (*4, 13*) indicates that the fast IIA MHC mRNA is present, albeit at different levels, in all normal skeletal muscles except the tensor fasciae latae (TFL). The presence of partially protected fragments is explained in the legend to Fig. 1B. This gene exhibits a particularly striking response to changes in thyroid hormone level, as the same hormonal stimulus produces completely opposite responses in its expression in different muscles. In hypothyroid animals, the fast IIA MHC gene is newly induced in the TFL but is deinduced in the soleus, while its level of expression does not change significantly in the diaphragm, masseter, and extensor digitorum longus (EDL). Furthermore, in hyperthyroid animals this gene is induced in the soleus, whereas it is deinduced to undetectable levels in the masseter and EDL but to a lesser degree in the diaphragm. Overall, the fast IIA MHC gene is up-regulated by thyroid hormone in the slow-twitch (soleus) muscle but down-regulated, with different degrees of sensitivity, in fast-twitch muscles.

Figure 1C shows the expression of the type IIB (fast glycolytic) MHC gene in five

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different muscles. The detection of a 304-nt fully protected fragment of clone pMHC62 (*4, 13*) indicates that this gene is expressed at high levels in most normal fast-twitch skeletal muscles and at low levels in the diaphragm and soleus. Hypothyroidism causes deinduction of this gene in the soleus, diaphragm, and masseter but not in the EDL and TFL. Hyperthyroidism slightly increases its expression in the soleus and diaphragm, while it does not cause significant changes in other muscles where its basal level of expression is already high. Overall, the fast IIB MHC gene appears to be responsive to thyroid hormone in some muscles (soleus, diaphragm, and masseter) but not in others.

Previous protein (*5*) and mRNA (*4, 14*) studies have demonstrated that, in the rat, myosin isozymes follow a sequential transition from embryonic to neonatal to adult form during skeletal muscle development, which is normally completed in the first few weeks of postnatal life. However, in newborn animals this developmental myosin transition can be modified by thyroid hormone (*15*). Furthermore, even in adult animals, the extraocular muscles coexpress six different MHC genes, including the embryonic and neonatal ones, both at the mRNA and protein levels (*13*). For these reasons, we next examined whether the embryonic and neonatal MHC genes could be reinduced in adult muscles subjected to hypo- or hyperthyroidism. Indeed, as shown in Fig. 2A, the expression of the embryonic MHC gene, detected by a fully protected 194-nt fragment of clone pMHC25 (*14*), is induced in the soleus and, to a lesser extent, in the diaphragm of hypothyroid animals but not in any other muscles examined. Similarly, expression of the neonatal MHC gene (Fig. 2B), identified by a 245-nt fully protected fragment of clone pFOD5 (*14*), is induced at a high level in the hypothyroid masseter but not in other muscles. It is of particular interest that the transition from the neonatal to the adult MHC's—a process highly responsive to thyroid hormone in the newborn (*15*)—is, once established, no longer reversible by hypothyroidism in the adult muscles examined, except in the masseter, where trace amounts of the neonatal MHC gene product are detectable in euthyroid animals (Fig. 2B). However, it is clear that these "developmental stage–specific" MHC genes can be reversibly modulated in specific adult muscles by changes in thyroid hormone level, further weakening the concept that these genes are under the strict control of a "developmental clock."

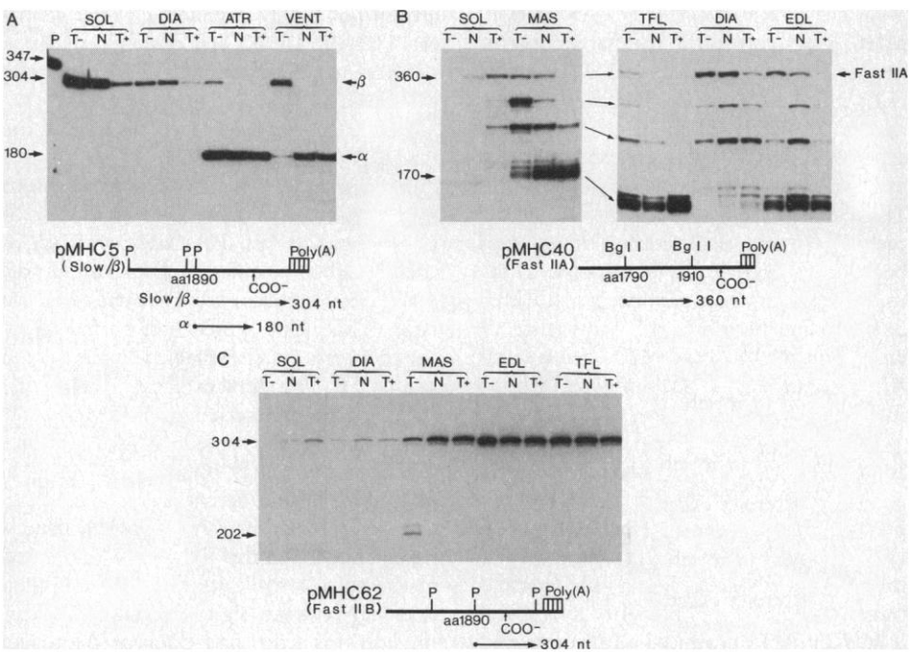


Fig. 1. Effect of thyroid hormone on the expression of the MHC genes. The animals used were 4-month-old male rats: hypothyroid (T−) (6 weeks after surgical thyroidectomy), euthyroid (N), and hyperthyroid (T+). Hyperthyroidism was induced by daily intraperitoneal injection of $T_3$ (5 μg per 100 g) for 8 days, long enough for the MHC mRNA to reach a new steady state after the hormonal switches, taking into account the half-life of the MHC mRNA (*9, 24*). The muscles obtained from each group of rats were cardiac ventricle (VENT) and atrium (ATR); skeletal slow-twitch muscle, soleus (SOL); and four skeletal fast-twitch muscles, diaphragm (DIA), masseter (MAS), TFL, and EDL. RNA extraction and S1 nuclease mapping were done as previously described (*9*) with 20 μg of total RNA and 150 U of S1 nuclease (New England Nuclear) per assay. (A) α- and β-MHC genes. The probe used was the 3′ end Pst I fragment of pCMHC5, a cDNA clone specific for the β-MHC gene (*9*). This 347-nt single-stranded probe contains 180 nt of common coding sequence at the carboxyl end of the α- and β-MHC, in addition to the entire 3′ untranslated sequence of the β-MHC gene, which diverges completely from the α-MHC gene. It also contains 43 nt of oligo-dT and -dG tails. (B) Type IIA (fast oxidative) MHC gene. The probe used was the single-stranded 360-nt Bgl I fragment of pMHC40, a cDNA clone specific for the fast IIA MHC gene (*4, 13*). This probe is composed entirely of coding sequence (coordinates 1410 to 1770). Because of the sequence conservation between coordinates 1410 and 1500 among various MHC cDNA clones, S1 nuclease mapping produces several partially protected fragments. Among these, the 170-nt fragment corresponds to the fast IIB MHC mRNA (*13*). The same fully protected pattern was observed by another probe composed entirely of 3′ untranslated sequences of this gene. (C) Type IIB (fast glycolytic) MHC gene. The probe used was the single-stranded 304-nt 3′ end Pst I fragment of pMHC-62, a cDNA clone specific for the fast IIB MHC gene (*4, 13*). This probe contains 215 nt of 3′ terminal coding sequence and the entire 89-nt 3′ untranslated sequences. It produces a 304-nt fully protected fragment when hybridized to the homologous (fast IIB MHC) mRNA. The numbers on the left indicate the size of the protected fragment in number of nucleotides. P, probe alone.

The results of the above S1 nuclease mapping analyses are summarized in Fig. 3. Expression of a given MHC gene and its response to thyroid hormone manipulation in the different muscles can be easily identified by following the columns horizontally. Similarly, the number of MHC genes and their relative levels of expression in a given muscle can be identified by following the columns vertically. Four general conclusions are readily drawn from these data: (i) more than one MHC gene is expressed in each anatomically defined muscle; (ii) each of six MHC genes analyzed is expressed in more than one muscle, but the "set" of MHC genes expressed and their relative levels of expression are characteristic of each muscle type, in agreement with previous studies detecting heterogeneity in the fiber composition of anatomically defined muscles (*2, 16*); (iii) all muscles tested respond to thyroid hormone manipulation; and (iv) all members of the MHC multigene family respond to thyroid hormone, but the mode of this response does not appear to be intrinsic to any given MHC gene but seems to be determined in a tissue-specific manner.

The tissue-specific response of each MHC gene is best exemplified by the α- and fast IIA MHC genes. The α-MHC gene is exquisitely sensitive to thyroid hormone in the ventricle but not at all in the atrium. Expression of the type IIA MHC gene is upregulated by thyroid hormone in slow skeletal muscle (soleus), whereas it is downregulated in fast skeletal muscles (dia-

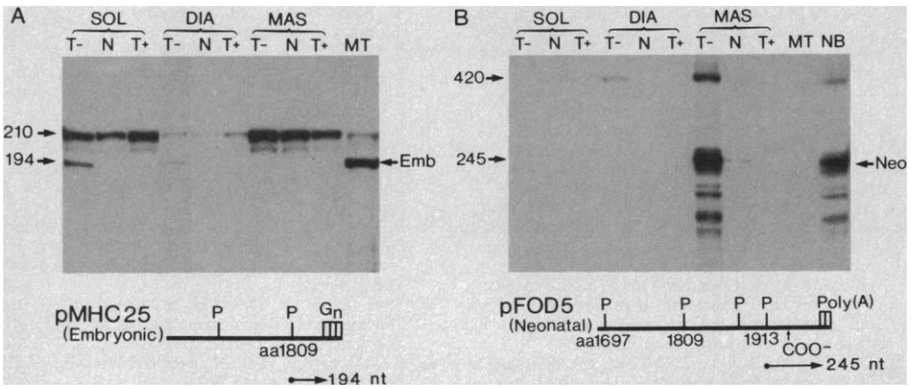


Fig. 2. Reinduction of the embryonic and neonatal skeletal MHC genes in adult muscles. (A) Embryonic MHC gene. The probe used was the 210-nt double-stranded Pst I fragment of pMHC25, a cDNA clone specific for the embryonic MHC gene (*14*). This probe, which covers the coding portion near the carboxyl terminal end of MHC (amino acid 1809 to 1873), contains 16 nt of oligo-dG tail and generates a fully protected fragment of 194 nt. The strong band of 210 nt seen throughout the gel represents undigested double-stranded probe. MT, differentiated $L_6E_9$ myotube RNA (*4*). (B) Neonatal MHC gene. The probe used was the double-stranded 420-nt Pst I–Bgl I fragment of pFOD5, a cDNA clone specific for the neonatal MHC (*14*). This fragment contains 105 nt of 3′ end terminal coding sequence, the entire 140-nt 3′ untranslated sequence, and a portion of pBR322. The fragment fully protected by the homologous (neonatal MHC) mRNA is 245 nt. NB, hind leg muscle RNA from newborn rats.

phragm, masseter, EDL, and TFL). To our knowledge, this is the first demonstration that the same gene responds in completely opposite directions to the same hormonal stimulus when expressed in different tissues. This complex pattern of regulation of a single MHC gene, in combination with the responsiveness to thyroid hormone exhibited by all the muscles tested, argues against a qualitative or quantitative difference in triiodothyronine ($T_3$) nuclear receptors in individual muscles as a sole determinant of tissue-specific regulation of the MHC genes. Despite this complexity, it is remarkable that, in a given muscle, hyperthyroidism either increases the mRNA levels of the MHC isoform associated with the higher velocity of fiber shortening and the higher myosin adenosinetriphosphatase activity or decreases the mRNA levels of the MHC isoform associated with the lower velocity of contraction. Hypothyroidism, on the other hand, effects the opposite changes. Although this analysis does not directly address the question of whether changes in the level of each specific mRNA caused by thyroid hormone take place in all or only a subset of the fibers in an anatomically defined muscle, complete replacement of MHC isoforms does occur in individual muscles during development, under pathological conditions, and in response to thyroid hormone (*5, 6, 9–11*).

The issue of whether thyroid hormone acts directly on the muscles or whether its effects are indirect (as by acting through changes in the pattern of nerve stimulation) cannot be resolved here. However, previous studies at the protein level involving myocyte culture (*17*), denervation (*18*), and the hereditary dwarf mouse (*19*) suggest that thyroid hormone, at least in the case of cardiac ventricle and fast skeletal muscle, acts directly on the muscle to regulate the MHC phenotype. As to the locus of thyroid hormone action in the cell, most of its characteristic biological effects appear to be mediated by an interaction of $T_3$ with specific nuclear receptors (*20*). In the best studied examples, such as the regulation of thyrotropin and growth-hormone synthesis, $T_3$ has been shown to act mainly at the transcriptional level (*21*). In the cardiac ventricle, kinetic studies of MHC mRNA accumulation during hormonal induction and deinduction (*9*) and nuclear runoff studies (*22*) suggest that modulation of the α- and β-MHC mRNA's by thyroid hormone is mainly regulated at the transcriptional level and not by changes in mRNA stability. It remains to be determined whether the same applies for hormone-dependent switches of the other MHC genes in different skeletal muscles.

In conclusion, the results presented here illustrate the highly complex regulation of the MHC multigene family. Each member of this multigene family can be under different developmental and hormonal regulatory programs, depending on the muscle in which it is expressed. Whatever the mechanisms involved in this tissue-specific regulation, they have to account for the fact that these gene switches take place in terminally differentiated cells in the absence of DNA replication (*23*). Although the available data do not allow us to further define the nature of this tissue-specific regulation, the fact that only certain MHC genes are expressed or inducible in different muscles suggests that events occurring during terminal differentiation of each muscle may determine which set of MHC genes is made potentially "available" for expression in a given muscle. In addition, the presence or absence of *cis*-acting regulatory sequences in each MHC gene could determine thyroid hormone responsiveness—either positive regulation, as in the case of the α- and fast IIB MHC genes, or negative, as in the case of the β-, embryonic, and neonatal MHC genes. This mode of regulation, however, is unlikely to fully account for the different patterns of developmentally and hormonally regulated expression of the same MHC gene in different muscles. Therefore, tissue-specific differences in nuclear $T_3$ receptors, local thyroid hormone metabolism, and secondary neuronal and hormonal signals induced by thyroid hormone may also play a role in determining regulation of the MHC multigene family in individual tissues.

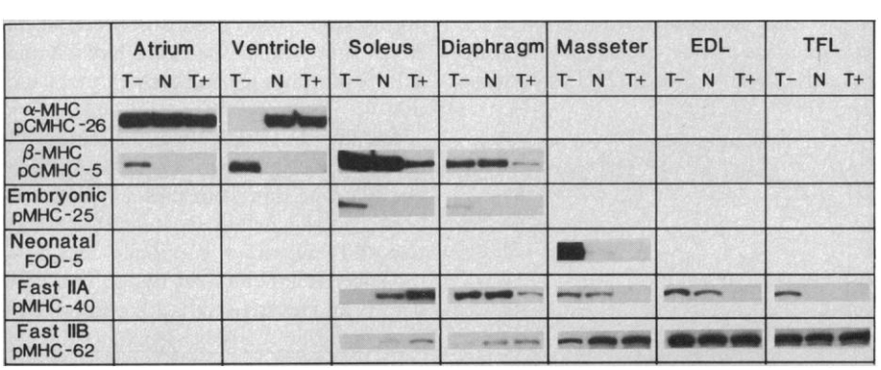


Fig. 3. Tissue-specific modulation of the MHC multigene family by thyroid hormone. The bands correspond to the fully protected fragments obtained by S1 nuclease mapping analysis (Figs. 1 and 2). The relative amount of each specific MHC mRNA present in the different muscles is reflected by the density of its corresponding band. Similar results were obtained by counting the radioactivity in the fully and partially protected bands detected by the different probes as described (*9*). A blank box indicates no detection of the corresponding MHC mRNA.

REFERENCES AND NOTES

1. M. Barany, *J. Gen. Physiol.* **50**, 197 (1967); K. Schwartz *et al.*, *J. Mol. Cell. Cardiol.* **13**, 1071 (1981).
2. R. G. Whalen *et al.*, in *Muscle Development: Molecular and Cellular Control*, M. L. Pearson and H. F. Epstein, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 25–33.
3. H. Czosnek *et al.*, *EMBO J.* **1**, 1299 (1982); L. A. Leinwand *et al.*, *Science* **221**, 766 (1983).
4. B. Nadal-Ginard *et al.*, in *Muscle Development: Molecular and Cellular Control*, M. L. Pearson and H. F. Epstein, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 143–168; V. Mahdavi *et al.*, in *Molecular Biology of Muscle Development*, C. Emerson, D. A. Fischman, B. Nadal-Ginard, M. A. Q. Siddiqui, Eds. (Liss, New York, in press).
5. R. G. Whalen *et al.*, *Nature (London)* **292**, 805 (1981).
6. N. A. Rubinstein *et al.*, *J. Cell Biol.* **79**, 252 (1978); F. Jolez and F. A. Sreter, *Annu. Rev. Physiol.* **43**, 531 (1981).
7. S. Salmons and F. A. Sreter, *Nature (London)* **263**, 30 (1976); A.-M. Lompre *et al.*, *ibid.* **282**, 105 (1979).
8. Y. Yazaki and M. S. Raben, *Circ. Res.* **36**, 208 (1975); D. Ianuzzo *et al.*, *Nature (London)* **270**, 74 (1977); J. F. Y. Hoh, P. A. McGrath, H. T. Hale, *J. Mol. Cell. Cardiol.* **10**, 1053 (1978); W. Dillman, *Diabetes* **29**, 579 (1980); E. Morkin *et al.*, *Prog. Cardiovasc. Dis.* **25**, 435 (1983).
9. A.-M. Lompre, B. Nadal-Ginard, V. Mahdavi, *J. Biol. Chem.* **259**, 6437 (1984); V. Mahdavi, A. P. Chambers, B. Nadal-Ginard, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2626 (1984).
10. A. M. Shinha *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5847 (1982).
11. A. M. Shinha *et al.*, *J. Biol. Chem.* **259**, 6674 (1984).
12. The nomenclature used for the different MHC mRNA's, cDNA clones, and corresponding genes follows the most common usage in the literature reflecting the developmental stage, muscle, or fiber type where their expression is predominant, without implying that their expression is restricted to these tissues or developmental stage.
13. D. F. Wieczorek *et al.*, *J. Cell Biol.* **101**, 618 (1985).
14. M. Periasamy, D. F. Wieczorek, B. Nadal-Ginard, *J. Biol. Chem.* **259**, 13,573 (1984); M. Periasamy *et al.*, *ibid.* **260**, 15,856 (1985).
15. B. Gambke *et al.*, *FEBS Lett.* **156**, 335 (1983); G. S. Butler-Browne, D. Herlicoviez, R. G. Whalen, *ibid.* **166**, 71 (1984).
16. G. F. Gauthier and S. Lowey, *J. Cell Biol.* **74**, 760 (1977); G. F. Gauthier *et al.*, *ibid.* **92**, 471 (1982).
17. A. C. Nag and M. Cheng, *Biochem. J.* **221**, 21 (1984); ——, R. Zak, *Exp. Cell Res.* **158**, 53 (1985).
18. L. Nwoye *et al.*, *Am. J. Physiol.* **242**, R401 (1982); E. C. B. Hall-Craggs, M. M. Wines, S. R. Max, *Exp. Neurol.* **80**, 252 (1983).
19. R. G. Whalen *et al.*, *J. Cell Biol.* **101**, 603 (1985).
20. J. H. Oppenheimer and H. H. Samuels, Eds., *Molecular Basis of Thyroid Hormone Action* (Academic Press, New York, 1983).
21. R. M. Evans, N. C. Birnberg, M. G. Rosenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7659 (1982); B. M. Yaffe and H. H. Samuels, *J. Biol. Chem.* **259**, 6284 (1984); M. A. Shupnick *et al.*, *ibid.* **260**, 2900 (1985).
22. P. K. Umeda *et al.*, in *Molecular Biology of Muscle Development*, C. Emerson, D. A. Fischman, B. Nadal-Ginard, M. A. Q. Siddiqui, Eds. (Liss, New York, in press).
23. F. E. Stockdale, *Dev. Biol.* **21**, 462 (1970); J. L. Doering and D. A. Fischman, *ibid.* **36**, 225 (1974); R. Zak, *Circ. Res.* **34** (Suppl. 2), II (1974).
24. R. M. Medford, H. T. Nguyen, B. Nadal-Ginard, *J. Biol. Chem.* **258**, 11,063 (1983).


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# Induction of HTLV-III/LAV from a Nonvirus-Producing T-Cell Line: Implications for Latency


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**When the human T-cell line A3.01 is infected with HTLV-III/LAV, the virus associated with the acquired immune deficiency syndrome (AIDS), most of the cells are killed. However, a small number of cells that lack the Leu-3 surface marker survive. Under normal conditions these surviving cells do not produce virus, nor can they be infected by the virus, but they can be induced to produce virus by treatment with 5-iodo-2′-deoxyuridine. This response can be induced for as long as 3 months after the initial infection, suggesting that the cells may harbor a latent form of HTLV-III/LAV.**


THE PRIMARY ETIOLOGIC AGENT OF the acquired immune deficiency syndrome (AIDS) has been identified as a human T-lymphotropic retrovirus (RV) that has been referred to as human T-lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), or AIDS-associated virus (*1–8*). When phytohemagglutinin-stimulated human lymphocytes are infected with this virus, the cells undergo a characteristic cytopathic effect that includes syncytia formation and, ultimately, cell death (*1, 2*). In contrast, a continuous lymphocyte line, H9, which has been used for the large-scale production of HTLV-III/LAV exhibits only modest cytopathic effects and no detectable cell death as a consequence of virus infection. We have recently described a variant of the CEM T-cell line A3.01, which is more than 95 percent susceptible to infection and undergoes all of the cytopathic changes associated with HTLV-III/LAV, including cell death (*9*). During the late stages of productive infection of A3.01 cells, a small percentage of the cells survive. These surviving cells lack the Leu-3 surface marker, and, although they do not produce the virus spontaneously, they clearly contain proviral DNA since infectious virus can be induced by treating the cells with 5-iodo-2′-deoxyuridine (IUdR). These findings have important implications in our understanding of the chronicity or possible latency of HTLV-III/LAV infection in humans and may serve as a useful model for investigations of the noncytocidal effects of the virus on human T-cell function.

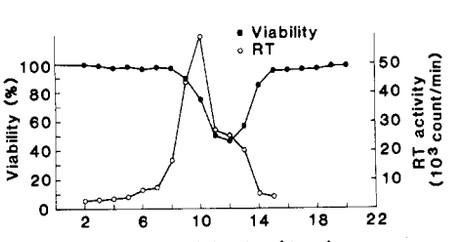


Fig. 1. Establishment of survivor NP cells. A3.01 cells ($1 \times 10^6$) were infected with the LAV isolate (*1*) of HTLV-III/LAV as previously described (*9*). Cell viability was determined by trypan blue exclusion.

Cells of the A3.01 line grow as two discrete populations (*9*). One, representing ~95 percent of the culture, expresses the Leu-3 (CD4, or T4) surface marker as well as Leu-1, class I HLA, and the transferrin receptor. The second population, comprising <5 percent of the culture, is Leu-3$^-$. Subclones of A3.01 cells, although initially Leu-3$^+$, consistently give rise to cells that make up the 2 to 5 percent of the culture that are Leu-3$^-$. One of these Leu-3$^-$ cells was cloned by limiting dilution (A2.01) and did not revert to the Leu-3$^+$ phenotype during an 8-month observation period. The A3.01 and A2.01 cells were indistinguishable by morphology and growth characteristics.

The HTLV-III/LAV was the same as that used in our original report on the A3.01 cell line (*9*). The time course of a typical infection is shown in Fig. 1. Reverse transcriptase (RT) activity was initially detected in the supernatant fluid on day 8, peaked on day 10, and reverted to background levels by


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