脊椎動物の時計遺伝子

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はじめに

高等動物のうち、遺伝学研究が最も進んでいる種は、マウスである。マウスにはさまざまな遺伝特性を持つ近交系やミュータント系が多数存在し、長年の遺伝学的データの蓄積に加え、取り扱い易さや比較的世代交代が早いことなどから、遺伝解析に最も適した哺乳動物として利用されている。特に最近では、マイクロサテライトなど DNA 多型マーカーが充実するなど連鎖解析技術の進歩に加え、YAC ライブラリーなどの開発による物理的地図作成技術の進歩、さらに、長年に渡る膨大な遺伝学データが蓄積し、インターネットを介して瞬時にデータ検索できる情報科学の進歩などにより、ますますマウスの重要性が増してきている。したがって、概日リズムに関する遺伝学研究もマウスを用いたものが多い。マウス以外の哺乳類ではハムスターやラットを用いた研究があるが、これらについては、マウスほど十分な遺伝学データの蓄積がないため解析が進んでいない。しかし、最近新しいマッピング解析技術として RLGS (restriction landmark genome scanning)法が開発され、ハムスターでもリンケージマップが作成されたことから(1)、今後これらの動物でも時計遺伝子の解析が可能となってゆくだろう。哺乳類以外の脊椎動物で注目すべきは、ゼブラフィッシュやメダカなどの魚類であろう。これらの魚は、体が小さいため飼育スペースを取らず、世代交代が速いうえ、大量に子孫を得ることができるので、突然変異誘発物質による時計遺伝子の分離も効率に行えるものと期待できる。しかし、問題はリズムの測定に関することで、遊泳行動ではそれほど明瞭なリズムが得られない。米国でゼブラフィッシュを用いた時計遺伝子の分離が計画されているが、この点の克服が成功への分かれ道である。

時計遺伝子は、ショウジョウバエの *per* や *timeless*、アカパンカビの *frq* など振動の発現に直接組み込まれていると考えられる遺伝子であるが、脊椎動物でこれに該当する遺伝子は見つかっていない。しかし、概日リズムに影響する遺伝子は多数存在する。従って、本稿では、これらの遺伝子を概日リズム関連遺伝子としてまとめた。

時計遺伝子へのアプローチ

突然変異により表れた表現型から、古典的メンデル遺伝に基づいた解析により遺伝子を同定することは、遺伝学の基本的手法である。これを Forward genetics と呼んでいる。一方、遺伝子工学や細胞工学の進歩に伴い、遺伝子を直接操作することができるようになり、遺伝子の働きや発現を変化させて表現型への影響を見ようとするいわゆるReverse Geneticsと呼ばれる手法が盛んに用いられるようになった。遺伝子ターゲッティングやアンチセンス DNA 法などがこれに当たる。また、概日リズムの基本的性質である、周期の温度補償性、光による位相反応性、周期が約 24 時間で持続する自律性などは全ての生物に共通しているが、このような時計の生理学的相同性から、概日リズム発現の分子機構に相同性が存在するものと考え、今までにクローニングされた時計遺伝子との相同遺伝子を検索する試みが行われている。これら以外

にも、Differential Display 法により概日リズムを示す mRNA を検出し遺伝子にアプローチする方法などが行なわれている。

Forward Genetics

Forward Geneticsはクラッシックな手法であるが、最近、SSLP (simple sequence length polymorphism)などのDNA 多型マーカーが多数マッピング(マウスで約 8000 のマーカーがマッピングされている)され、また、マウスの種間・亜種間交配を利用して詳細な連鎖解析が可能になるなどの連鎖解析技術の進歩やポジショナルクローニング技術の進歩、さらに、マウスデータベースの充実などにより、ネオクラッシックと呼ばれる様に Forward Genetics の進展には著しいものがある。Forward Genetics を用いた時計遺伝子へのアプローチは、まず、概日リズムの突然変異を見いだすことからはじまるが、現在までに得られた突然変異遺伝子は、化学変異原物質(ENU:N-エチル-ニトロソ尿素など)を用いて人為的に誘発したものと、自然発症したものとに分けることができる。前者には、マウスの *Clock*(2)、*Whl*(3)があり染色体上へのマッピングが完了している。*Clock* は半優性型の遺伝をし、ヘテロ接合体では周期が 24 時間より長く(野生型は24時間より短い)、ホモ接合体では最初 27-28 時間の極端に長い周期を示した後リズムが消失する。*Whl* も同様に半優性遺伝子で、周期が24時間より長くなる。また、この突然変異マウスは回転行動などの異常行動を示し、さらに光に対する反応性にも異常が認められる。自然発症した突然変異では、ハムスターで発見された*tau*突然変異遺伝子がある(4)。これは哺乳類で最初に発見された概日リズム突然変異遺伝子で、野生型のハムスターの周期はほぼ 24 時間に近いが、この遺伝子をヘテロ接合体で持つと周期が22時間となり、ホモ接合体では 20 時間となる。*tau* 突然変異ハムスターは、概日リズムの生理機構解明のために利用され成果をあげているが、マウスのような遺伝学的手法が使えないため、今のところ遺伝子クローニングなどへの研究の進展はない。

Forward Genetics を使ったもう一つのアプローチは、既存の系統を使った QTL (Quantitative trait locus)解析である。QTL 法は、量的形質に関連する複数の遺伝子座をマッピングする方法として新しく登場した(5)。一般に、行動などの形質は複数の遺伝子が関与する量的形質としてとらえられるが、従来は、このような複数の遺伝子が関与する形質を遺伝子レベルで解析することが困難であった。しかし、最近の分子遺伝学的技術の向上に伴い、高密度な遺伝子地図が作出されたことやデータ処理における統計方法の改良などにより、マウスでQTL解析を行うことが可能となった。QTL 解析を行った研究はまだわずかであるが、色々な系統を用いた QTL 解析により、時計遺伝子が存在する複数の候補遺伝子座領域が明らかになってくるであろう。本稿では、QTL 解析に必要な情報として概日リズムに関する遺伝的差について報告した論文も含めた。

Reverse Genetics

特定の遺伝子の機能を欠失させたり、過剰発現させたりして遺伝子の機能を個体レベルで解析しようとする Reverse Genetics は、遺伝子産物の生体内での機能を解析する有効な手段として広く用いられている。概日リズムに関しても、特定の遺伝子産物を欠失させるノックアウトマウスを用いた研究がいくつかの研究室で行なわれているが、発表されているものは多くない。この様なジーンターゲッティング法は、特定の機能分子の生体内での役割を探るうえで有効な手段となることは間違いないが、一方で、せっかく作っても他の遺伝子が機能を代償してしまうなどの理由で、表現型が野生型と変わらないこともよくあると言われている。高等動物の概日リズムに関しては、今のところ時計遺伝子

が分かっていないが、候補遺伝子が見つかれば、原因遺伝子と見極めるために行うrescue実験として重要である。

相同遺伝子の検索

今までにクローニングされている時計遺伝子は、ショウジョウバエの *per*、*timeless*、アラビドプシスの *toc* とアカパンカビの *frq*,シアノバクテリアの三つの ORF(D,E,F)であるが、これらの間には相同性が見られない。高等動物では、齧歯類において相同性遺伝子の検索を *per* 遺伝子について調べた報告がいくつかある。

振動体組織における時計関連遺伝子

脊椎動物の概日系は生物時計本体である振動体と、外界からの情報を振動体に伝える入力系、振動体の時間情報を様々な生理リズムに発現する出力系に分けることができる。振動体は、種によっても異なるが、一般に、網膜、松果体、視交差上核に存在すると言われている。本稿では、これらの組織について、概日リズムに関連する遺伝子や遺伝子発現などを扱ったものをそれぞれの機能分子ごとにまとめた。

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A Forward Genetics

1.遺伝的差(系統差)

Mouse

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2.QTL 解析

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B Reverse Genetics

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D 振動体組織における時計関連遺伝子

PINEAL AND EYE

Photopigment

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