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(54)名稱

調控核受體之人工轉錄因子及其治療用途

ARTIFICIAL TRANSCRIPTION FACTORS REGULATING NUCLEAR RECEPTORS AND THEIR THERAPEUTIC USE

(57)摘要

本發明係關於一種人工轉錄因子，其包含與抑制性或活化性蛋白質域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啟動子區域為目標之多指鋅指蛋白。在特定實例中，核受體基因之此等啟動子區域調節糖皮質激素受體、雄激素受體或雌激素受體 ESR1 之表現。針對該糖皮質激素受體之人工轉錄因子適用於治療由糖皮質激素調節之疾病，諸如發炎性過程、糖尿病、肥胖症、冠狀動脈病、哮喘、乳糜瀉及紅斑狼瘡。針對該雄激素受體之人工轉錄因子適用於治療由睪固酮調節之疾病，諸如各種癌症、冠狀動脈病、代謝失調(諸如肥胖症或糖尿病)或情感障礙(諸如精神分裂症、抑鬱症或注意力不足過動症)。針對該雌激素受體之人工轉錄因子適用於治療由雌激素調節之疾病，諸如各種癌症、心血管疾病、骨質疏鬆症或情感障礙。

The invention relates to an artificial transcription factor comprising a polydactyl zinc finger protein targeting specifically a promoter region of a nuclear receptor gene fused to an inhibitory or activatory protein domain, a nuclear localization sequence, and a protein transduction domain. In particular examples these promoter regions of a nuclear receptor gene regulate the expression of the glucocorticoid receptor, the androgen receptor, or the estrogen receptor ESR1. Artificial transcription factors directed against the glucocorticoid receptor are useful in the treatment of diseases modulated by glucocorticoids, such as inflammatory processes, diabetes, obesity, coronary artery disease, asthma, celiac disease and lupus erythematosus. Artificial transcription factors directed against the androgen receptor are useful in the treatment of diseases modulated by testosterone, such as various cancers, coronary artery disease, metabolic disorders such as obesity or diabetes or mood disorders such as schizophrenia, depression or attention deficit hyperactivity disorder. Artificial transcription factors directed against the estrogen receptor are useful in the treatment of diseases modulated by estrogens, such as various cancers, cardiovascular disease, osteoporosis or mood disorders.

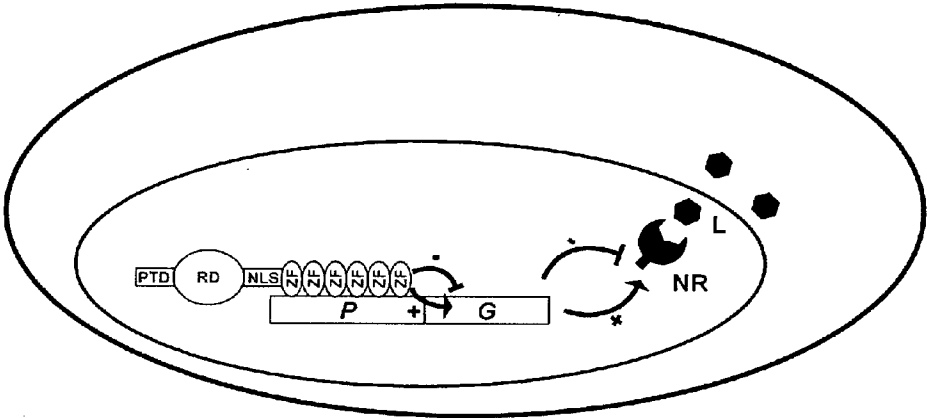


圖1

發明摘要

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【發明名稱】(中文/英文)

調控核受體之人工轉錄因子及其治療用途

Artificial transcription factors regulating nuclear receptors and their therapeutic
use

【中文】

本發明係關於一種人工轉錄因子，其包含與抑制性或活化性蛋白質域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啟動子區域為目標之多指鋅指蛋白。在特定實例中，核受體基因之此等啟動子區域調節糖皮質激素受體、雄激素受體或雌激素受體 ESR1 之表現。針對該糖皮質激素受體之人工轉錄因子適用於治療由糖皮質激素調節之疾病，諸如發炎性過程、糖尿病、肥胖症、冠狀動脈病、哮喘、乳糜瀉及紅斑狼瘡。針對該雄激素受體之人工轉錄因子適用於治療由睪固酮調節之疾病，諸如各種癌症、冠狀動脈病、代謝失調（諸如肥胖症或糖尿病）或情感障礙（諸如精神分裂症、抑鬱症或注意力不足過動症）。針對該雌激素受體之人工轉錄因子適用於治療由雌激素調節之疾病，諸如各種癌症、心血管疾病、骨質疏鬆症或情感障礙。

【英文】

The invention relates to an artificial transcription factor comprising a polydactyl zinc finger protein targeting specifically a promoter region of a nuclear receptor gene fused to an inhibitory or activatory protein domain, a nuclear localization sequence, and a protein transduction domain. In particular examples these promoter regions of a nuclear receptor gene regulate the expression of the glucocorticoid receptor, the androgen receptor, or the estrogen receptor ESR1. Artificial transcription factors directed against the glucocorticoid receptor are useful in the treatment of diseases modulated by glucocorticoids, such as inflammatory processes, diabetes, obesity, coronary artery disease, asthma, celiac disease and lupus erythematosus. Artificial transcription factors directed against the androgen receptor are useful in the treatment of diseases modulated by testosterone, such as various cancers, coronary artery disease, metabolic disorders such as obesity or diabetes or mood disorders such as schizophrenia, depression or attention deficit hyperactivity disorder. Artificial transcription factors directed against the estrogen receptor are useful in the treatment of diseases modulated by estrogens, such as various cancers, cardiovascular disease, osteoporosis or mood disorders.

✓
【代表圖】

【本案指定代表圖】：第（ 1 ）圖。

【本代表圖之符號簡單說明】：

無

【本案若有化學式時，請揭示最能顯示發明特徵的化學式】：

無

發明專利說明書

(本說明書格式、順序，請勿任意更動)

【發明名稱】(中文/英文)

調控核受體之人工轉錄因子及其治療用途

Artificial transcription factors regulating nuclear receptors and their therapeutic use

【技術領域】

本發明係關於人工轉錄因子，其包含與抑制性或活性域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啓動子區域爲目標之多指鋅指蛋白，及其於治療由該等核受體之活性引起或調節之疾病中的用途。

【先前技術】

人工轉錄因子 (artificial transcription factor, ATF) 被提議爲適用於調控基因表現之工具 (Sera T., 2009, *Adv Drug Deliv Rev* 61, 513-526)。經由抑制或活化基因轉錄而影響表現之許多天然存在之轉錄因子具有用於識別特定 DNA 序列之複雜的特異性域。若意欲調節其特異性且以基因爲目標，則此使其成爲無吸引力的操作目標。然而，一類特定轉錄因子含有若干個所謂的鋅指 (zinc finger, ZF) 域，其爲模組化的且因此有助於遺傳工程改造。鋅指爲以幾乎獨立的三個 DNA 鹼基對爲目標的短 (30 個胺基酸) DNA 結合基元。因此含有融合在一起之若干個鋅指的蛋白質能夠識別較長 DNA 序列。六聚鋅指蛋白 (zinc finger protein, ZFP) 識別 18 個鹼基對 (bp) DNA 目標，其在整個人類基因組中幾乎爲唯一的。最初認爲是完全情境獨立的，更多深入分析揭露對於鋅指之某種情境特異性 (Klug A., 2010, *Annu Rev Biochem* 79, 213-231)。使鋅指識別表面中之某些胺基酸突變，改變 ZF 模組

之結合特異性產生為 5'-GNN-3'、5'-CNN-3'、5'-ANN-3'及一些 5'-TNN-3'密碼子中之大部分所定義之 ZF 構建嵌段（例如所謂的巴巴斯模組（Barbas module），參見 Gonzalez B., 2010, *Nat Protoc* 5, 791-810）。雖然對人工轉錄因子之早期工作集中於基於將預選鋅指與已知 3 bp 目標序列組合之合理設計，但鋅指之某種情境特異性的實現需要產生大的鋅指文庫，使用諸如細菌或酵母單雜交、噬菌體呈現、隔室化核糖體呈現或使用 FACS 分析之活體內選擇之先進方法來查詢該等文庫。

使用該等人工鋅指蛋白，可以高特異性以人類基因組中之 DNA 基因座為目標。因此，此等鋅指蛋白為將具有轉錄調控活性之蛋白質域運輸至特定啟動子序列，從而使得所關注基因之表現得到調控的理想工具。適於轉錄沉默之域為作為 N 末端（SEQ ID NO: 1）或 C 末端（SEQ ID NO: 2）KRAB 域之 Krueppel 相關域（Krueppel-associated domain, KRAB）、Sin3 相互作用域（SID, SEQ ID NO: 3）及 ERF 抑制蛋白域（ERD, SEQ ID NO: 4），而基因轉錄之活化經由疱疹病毒單純形 VP16（SEQ ID NO: 5）或 VP64（VP16 之四聚重複，SEQ ID NO: 6）域來達成（Beerli R.R.等人, 1998, *Proc Natl Acad Sci USA* 95, 14628-14633）。認為賦予轉錄活化之其他域為 CJ7（SEQ ID NO: 7）、p65-TA1（SEQ ID NO: 8）、SAD（SEQ ID NO: 9）、NF-1（SEQ ID NO: 10）、AP-2（SEQ ID NO: 11）、SP1-A（SEQ ID NO: 12）、SP1-B（SEQ ID NO: 13）、Oct-1（SEQ ID NO: 14）、Oct-2（SEQ ID NO: 15）、Oct-2_5x（SEQ ID NO: 16）、MTF-1（SEQ ID NO: 17）、BTEB-2（SEQ ID NO: 18）及 LKLF（SEQ ID NO: 19）。另外，認為由基因本體論 GO:0001071（http://amigo.geneontology.org/cgi-bin/amigo/term_details?term=GO:0001071）定

義之蛋白質之轉錄活性域達成目標蛋白質之轉錄調控。包含經工程改造之鋅指蛋白以及調控域之融合蛋白被稱為人工轉錄因子。

雖然由於特定特點之高保守，小分子藥並不總是能夠選擇性地以特定蛋白質家族之某一成員為目標，但基於天然存在或經工程改造之蛋白質之生物製品提供如所示對於基於抗體之新穎藥之良好特異性。然而，迄今為止，幾乎所有生物製品均在細胞外起作用。尤其上文所提及之人工轉錄因子將適於以治療上有用之方式影響基因轉錄。然而，將該等因子傳遞至作用位點（核）並不容易達成，因此阻礙了治療性人工轉錄因子方法之可用性，例如藉由依靠反轉錄病毒傳遞，其具有此方法之所有缺點，諸如免疫原性及細胞轉型潛力（Lund C.V.等人, 2005, *Mol Cell Biol* 25, 9082-9091）。

顯示所謂的蛋白轉導域（protein transduction domain, PTD）促進蛋白質跨越質膜位移至胞質液/核質中。顯示諸如 HIV 衍生 TAT 肽 (SEQ ID NO: 20) 等短肽誘導貨物蛋白質之細胞類型獨立性大吞飲泡攝取（Wadia J.S.等人, 2004, *Nat Med* 10, 310-315）。在到達胞質液時，顯示融合蛋白具有生物活性。有趣的是，甚至錯誤摺疊之蛋白質可在蛋白質轉導後很可能經由細胞內伴侶蛋白之作用變得具有功能。

核受體為配位體活化之轉錄因子的蛋白質超家族。不同於大多數其他細胞膜錨定受體，其為定位於胞質液或核質之可溶性蛋白質。在配位體結合及隨後二聚化時，核受體能夠經由 DNA 結合及基因表現之調節來充當轉錄因子。核受體之配位體為親脂性分子，尤其為類固醇及甲狀腺激素、脂肪酸及膽汁酸、視黃酸、維生素 D3 及前列腺素（McEwan I.J., *Methods in Molecular Biology: The Nuclear Receptor Superfamily*, 505, 3-17）。在配位體結合

時，核受體二聚化，由此觸發與配位體反應性基因啓動子內之特定轉錄因子特異性 DNA 反應元件內部結合，從而引起基因表現之活化或抑制。假定核受體負責調控許多廣泛作用激素（諸如類固醇）及重要代謝產物之活性，核受體之錯誤功能及機能障礙與許多病症之自然史有關。

將使用促效劑或拮抗劑調控核受體之活性用於治療性目的。使用諸如促效性地塞米松（dexamethasone）之皮質類固醇調控糖皮質激素受體（GR）功能為用於影響發炎性疾病之常見臨床實踐。核受體活性之另一調控以口服避孕藥來例示，其中使用雌激素受體（ESR1/ER）及孕酮受體之活化來防止女性卵子受精。在另一個實例中，使用諸如氟他胺（flutamide）或比卡魯胺（bicalutamide）之抗雄激素阻斷雄激素受體（AR）證明適用於治療 AR 依賴性前列腺癌。此外，藉由阻斷雌激素合成及因此雌激素之可用性來阻斷雌激素受體為女性乳癌或男性之男性乳腺增生之標準療法。

【發明內容】

本發明係關於一種人工轉錄因子，其包含與抑制性或活化性蛋白質域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啓動子區域為目標之多指鋅指蛋白，及包含該人工轉錄因子之醫藥組成物。此外，本發明係關於該等人工轉錄因子用於調節細胞對核受體配位體之反應及治療藉由特定效應物與該等核受體之結合調節之疾病的用途。

在一特定具體實例中，核受體基因之啓動子區域為雄激素受體啓動子（SEQ ID NO: 21）。在此特定具體實例中，本發明係關於一種以雄激素受體啓動子為目標之人工轉錄因子，其用於影響細胞對睪固酮之反應，用於降低或增加雄激素受體含量，及用於治療由睪固酮調控之疾病。同樣，本發

明係關於一種治療由睪固酮調控之疾病的方法，其包含向有需要之患者投予治療有效量之以雄激素受體啟動子為目標的本發明之人工轉錄因子。

在另一特定具體實例中，核受體基因之啟動子區域為雌激素受體啟動子 (SEQ ID NO: 22)。在此特定具體實例中，本發明係關於以雌激素受體啟動子為目標之該人工轉錄因子，其用於影響細胞對雌激素之反應，用於降低或增加雌激素受體含量，及用於治療由雌激素調控之疾病。同樣，本發明係關於一種治療由雌激素調控之疾病的方法，其包含向有需要之患者投予治療有效量之以雌激素受體啟動子為目標的本發明之人工轉錄因子。

在另一特定具體實例中，核受體基因之啟動子區域為糖皮質激素受體啟動子 (SEQ ID NO: 21)。在此特定具體實例中，本發明係關於一種以糖皮質激素受體啟動子為目標之人工轉錄因子，其用於影響細胞對糖皮質激素之反應，用於降低或增加糖皮質激素受體含量，及用於治療由糖皮質激素調控之疾病，尤其用於治療由糖皮質激素調控之眼病。同樣，本發明係關於一種治療由糖皮質激素調控之疾病的方法，其包含向有需要之患者投予治療有效量之以糖皮質激素受體啟動子為目標的本發明之人工轉錄因子。

本發明進一步係關於編碼本發明之人工轉錄因子的核酸，包含此等核酸之載體，及包含該等載體之宿主細胞。

【圖式簡單說明】

圖 1：使用可轉導人工轉錄因子調控基因表現

藉由蛋白轉導域 (protein transduction domain, PTD) (諸如 TAT 或其他域) 之作用將含有與抑制性/活性域 (RD = 調控域) 以及核定位序列 (NLS) 融合之特異性以核受體基因 (G) 之啟動子 (P) 區域為目標之六聚鋅指 (ZF)

蛋白的人工轉錄因子運輸至細胞中。視轉錄調控域而定，受體基因表現增加(+)或抑制(-)，導致核受體(NR)之表現增強或減弱及因此細胞對核受體配位體(L)之反應增強或減弱。

圖 2：人類糖皮質激素受體啓動子及人工轉錄因子目標位點

展示糖皮質激素受體啓動子之 5'未轉譯區域 (SEQ ID NO: 21)。突出本發明之人工轉錄因子的轉錄起始位點 (標記爲粗體，位置 707) 及三個結合位點 (加下劃線)。

圖 3：人類雄激素受體啓動子及人工轉錄因子目標位點

展示雄激素受體啓動子之 5'未轉譯區域 (SEQ ID NO:22)。突出本發明之人工轉錄因子的轉錄起始位點 (標記爲粗體，位置 768) 及四個結合位點 (加下劃線)。

圖 4：人類雌激素受體啓動子及人工轉錄因子目標位點

展示雌激素受體啓動子之 5'未轉譯區域 (SEQ ID NO: 23)。突出本發明之人工轉錄因子的轉錄起始位點 (標記爲粗體，位置 960) 及三個結合位點 (加下劃線)。

圖 5：在螢光素酶報導分子分析中 AR4 rep 能夠抑制基因表現

在 OptiMEM 培養基中用 1 μ m AR4rep 處理含有由在雜交 CMV/AR_TS4 啓動子控制下之高斯椰屬 (*Gaussia*) 螢光素酶以及在組成性 CMV 啓動子控制下之分泌型鹼性磷酸酶組成之報導分子構築體的 HEK 293 FlpInTRex 細胞 2 小時。用無關人工轉錄因子 ATFControl (SEQ ID NO: 24) 處理用作對照組 (標記爲 c)。在 AR4rep 處理後 24 小時量測螢光素酶以及分泌型鹼性磷酸酶。校正爲分泌型鹼性磷酸酶之螢光素酶活性表示爲以對照組之百分比計

的相對螢光素酶活性(RLA)。使用雙尾、未配對的學生 t 檢驗(Student's t-test)分析統計顯著性。P 值< 0.01 以**標記。

【實施方式】

本發明係關於一種人工轉錄因子，其包含與抑制性或活化性蛋白質域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啟動子區域為目標之多指鋅指蛋白，及包含該人工轉錄因子之醫藥組成物。

與錨定膜且包含或含有跨膜蛋白之幾乎所有其他細胞受體相反，核受體為在一個多肽中併有配位體結合及轉錄因子活性之可溶性蛋白。核受體定位於胞質液或核質，其中其在配位體結合時活化，二聚化且變成調控一系列巨大轉錄程式之活性轉錄因子。不同於上文所提及之結合細胞外之配位體且將信號轉導穿過質膜至細胞中的膜錨定受體，核受體結合能夠穿過質膜以接近其同源受體之親脂性配位體。另外，在達成所欲細胞結果之前，大多數膜結合受體依靠複雜的信號放大機制。另一方面，核受體直接將配位體結合轉化為細胞反應。

許多疾病之治療基於調控核受體信號傳導。實例為糖皮質激素活化糖皮質類固醇受體之發炎過程、雄激素受體之拮抗劑具有有益治療作用之前列腺癌或阻斷雌激素受體信號傳導證明有用之乳癌。傳統上，出於治療目的，使用核受體促效劑或拮抗劑形式之小分子來影響受體信號傳導。然而，核受體信號傳導亦可受核受體蛋白表現之直接調控影響，且該調控為本發明之本題。

本發明中所考慮之核受體為由以下人類基因編碼之人類核受體：*AR*、*ESR1*、*ESR2*、*ESRRA*、*ESRRB*、*ESRRG*、*HNF4A*、*HNF4G*、*NR0B1*、*NR0B2*、

NR1D1、*NR1D2*、*NR1H2*、*NR1H3*、*NR1H4*、*NR1I2*、*NR1I3*、*NR2C1*、*NR2C2*、*NR2E1*、*NR2E3*、*NR2F1*、*NR2F2*、*NR2F6*、*NR3C1*、*NR3C2*、*NR4A1*、*NR4A2*、*NR4A3*、*NR5A1*、*NR5A2*、*NR6A1*、*PGR*、*PPARA*、*PPARD*、*PPARG*、*RARA*、*RARB*、*RARG*、*RORA*、*RORB*、*RORC*、*RXRA*、*RXRB*、*RXRG*、*THRA*、*THRB* 及 *VDR*。

另外考慮由與所提及之人類核受體基因有關之基因編碼之非人類核受體，例如豬、馬、牛、貓、犬或鼠轉錄因子。

根據目前技術水平，使用病毒轉導完成人工轉錄因子之細胞內表現。病毒載體具有格外高的免疫原性潛力，由此限制其在某種治療之重複應用中之使用。由於鋅指模組之高保守，根據本發明之人工轉錄因子的應用，該免疫反應將為微小的或不存在，或可藉由總體結構之小變化而避免或進一步減至最少，從而消除免疫原性，同時仍保留目標位點結合及由此保留功能。此外，認為用聚乙二醇修飾本發明之人工轉錄因子可減少免疫原性。另外，將本發明之人工轉錄因子施用於免疫特權器官（諸如眼睛及腦）將避免任何免疫反應，且誘導對人工轉錄因子之全身耐受性。為了治療免疫特權器官外之慢性疾病，考慮經由先前眼內注射誘導免疫耐受性。

傳統上用作治療劑混合物之幾類小分子並不適於以基因表現之調節為目標。因此，許多有前途的藥物目標及相關疾病並不適合典型藥物方法。此對於認視不可製成藥物之轉錄因子而言尤其如此。相比之下，本發明之人工轉錄因子均屬於具有高度定義總組成之同一物質類別。以兩種非常不同的啟動子序列為目標之兩種基於六聚鋅指蛋白之人工轉錄因子仍具有85%之最小胺基酸序列一致性及總體類似三級結構，且可經由標準化方法

(如下文所述)以快速且經濟的方式產生。因此，本發明之人工轉錄因子將對於一組廣泛且不同的目標之格外高特異性與總體類似組成組合在一類分子中。另外，將本發明之人工轉錄因子調配至藥物中可依靠先前經驗，從而進一步加快藥物開發過程。

蛋白轉導域(PTD)介導之人工轉錄因子之細胞內傳遞為以新穎方式利用生物製品之高選擇性以受體分子為目標之新方式。雖然習知藥物調節某些受體之活性，但人工轉錄因子改變此等蛋白質之可用性。且因為人工轉錄因子經定製以特異性作用於該等受體基因之啟動子區域，所以本發明允許選擇性地以密切相關蛋白質為目標。此僅基於密切相關蛋白質之啟動子區域的寬鬆保守。蛋白轉導域介導之人工轉錄因子傳遞適用於調節細胞核受體配位體之反應。

所考慮之蛋白轉導域為 HIV TAT、肽 mT02 (SEQ ID NO: 25)、肽 mT03 (SEQ ID NO: 26)、R9 肽 (SEQ ID NO: 27)、ANTP 域 (SEQ ID NO: 28) 或能夠運輸貨物穿過質膜之其他肽。

本發明亦關於該等人工轉錄因子於治療藉由核受體配位體與核受體之結合調節之疾病中的用途，其中多指鋅指蛋白特異性以核受體基因之啟動子區域為目標。同樣，本發明係關於一種治療疾病之方法，其包含向有需要之患者投予治療有效量之人工轉錄因子，其中該待治療之疾病藉由特定效應物與核受體之結合調節，其中多指鋅指蛋白特異性以受體基因啟動子為目標。

所考慮之多指鋅指蛋白為四聚、五聚、六聚或七聚鋅指蛋白。「四聚」、「五聚」、「六聚」及「七聚」意謂鋅指蛋白分別由四、五、六及七個部分蛋

白質結構組成，其中每一者對於特定核苷酸三聯體具有結合特異性。較佳地，人工轉錄因子包含六聚鋅指蛋白。

特定啟動子區域內之目標位點的選擇

目標位點選擇對於成功產生功能性人工轉錄因子至關重要。對於活體內調節核受體基因表現之人工轉錄因子，其必須在核受體基因之基因組環境下結合其目標位點。此需要 DNA 目標位點之可接近性，意謂區域中之染色體 DNA 未在組蛋白周圍緊密包裹為核小體且（諸如甲基化）DNA 修飾不會干擾人工轉錄因子結合。雖然人類基因組之大部分緊密包裹且無轉錄活性，但主動轉錄基因之轉錄起始位點（-1000 至+200bp）的附近對於內源性轉錄因子及轉錄機構（諸如 RNA 聚合酶）必須為可接近的。因此，選擇任何特定目標基因之此區域中之目標位點將大大提高產生具有所需活體內功能之人工轉錄因子的成功率。

人類糖皮質激素、雄激素及雌激素受體基因啟動子內之目標位點的選擇

針對具有(G/C/ANN)₆之一般組成的潛在 18 bp 目標位點之存在分析包含包括人類糖皮質激素、雄激素及雌激素受體開放閱讀框（圖 2、3 及 4）之轉錄起始位點之 1000 bp 的啟動子區域，其中 G 為核苷酸鳥嘌呤，C 為核苷酸胞嘧啶，A 為核苷酸腺嘌呤且 N 代表四種核苷酸鳥嘌呤、胞嘧啶、腺嘌呤及胸腺嘧啶中之每一者。基於相對於轉錄起始位點之位置選擇每一啟動子中之三至四個目標位點。存在於糖皮質激素受體基因啟動子中之目標位點為 GR_TS1 (SEQ ID NO: 29)、GR_TS2 (SEQ ID NO: 30)、GR_TS3 (SEQ ID NO: 31)，存在於雄激素受體中之目標位點為 AR_TS1 (SEQ ID NO: 32)、

AR_TS2 (SEQ ID NO: 33)、AR_TS3 (SEQ ID NO: 34) 及 AR_TS4 (SEQ ID NO: 35)。雌激素受體基因啟動子中所鑑別之目標位點為 ER_TS1 (SEQ ID NO: 36)、ER_TS2 (SEQ ID NO: 37) 及 ER_TS3 (SEQ ID NO: 38)。亦考慮選自轉錄起點上游 2000 bp 之糖皮質激素受體、雌激素受體及雄激素受體之調控區域的具有一般組成(G/C/ANN)₅ 及(G/C/ANN)₆之目標位點。

以糖皮質激素受體啟動子為目標之可轉導人工轉錄因子

特定六聚鋅指蛋白由使用 ZiFit 軟體 v3.3 (Sander J.D., *Nucleic Acids Research* 35, 599-605) 之所謂巴巴斯鋅指模組設置 (Gonzalez B., 2010, *Nat Protoc* 5, 791-810) 構成。為了產生以糖皮質激素受體為目標之活性可轉導人工轉錄因子，使六聚鋅指蛋白以 GR_TS1 為目標之 ZFP-GR1 (SEQ ID NO: 39)、以 GR_TS2 為目標之 ZFP-GR2 (SEQ ID NO: 40) 及以 GR_TS3 為目標之 ZFP-GR3 (SEQ ID NO: 41) 與蛋白轉導域 TAT 以及轉錄活化域 VP64 融合，得到人工轉錄因子 GR1akt (SEQ ID NO: 42)、GR2akt (SEQ ID NO: 43) 及 GR3akt (SEQ ID NO: 44)。為了產生具有負調控活性之可轉導人工轉錄因子，使六聚鋅指蛋白 ZFP-GR1 至 ZFP-GR3 與蛋白轉導域 TAT 以及轉錄抑制域 KRAB 融合，得到人工轉錄因子 GR1rep (SEQ ID NO: 45)、GR2rep (SEQ ID NO: 46) 及 GR3rep (SEQ ID NO: 47)。

以雄激素受體啟動子為目標之可轉導人工轉錄因子

特定六聚鋅指蛋白由使用 ZiFit 軟體 v3.3 之所謂巴巴斯鋅指模組設置構成。使用酵母單雜交篩選選擇以 AR 啟動子為目標之其他鋅指蛋白。為了產生以雄激素受體為目標之活性可轉導人工轉錄因子，使六聚鋅指蛋白以 AR_TS1 為目標之 ZFP-AR1 (SEQ ID NO: 48)、以 AR_TS2 為目標之 ZFP-AR2

(SEQ ID NO: 49)、以 AR_TS3 爲目標之 ZFP-AR3 (SEQ ID NO: 50) 及以 AR_TS4 爲目標之 ZFP-AR4 (SEQ ID NO: 51)、ZFP-AR5 (SEQ ID NO: 52) 及 ZFP-AR6 (SEQ ID NO: 53) 與蛋白轉導域 TAT 以及轉錄活化域 VP64 融合，得到人工轉錄因子 AR1akt (SEQ ID NO: 54)、AR2akt (SEQ ID NO: 55)、AR3akt (SEQ ID NO: 56)、AR4akt (SEQ ID NO: 57)、AR5akt (SEQ ID NO: 58) 及 AR6akt (SEQ ID NO: 59)。爲了產生具有負調控活性之可轉導人工轉錄因子，使六聚鋅指蛋白 ZFP-AR1 至 ZFP-AR6 與蛋白轉導域 TAT 以及轉錄抑制域 SID 融合，得到人工轉錄因子 AR1rep (SEQ ID NO: 60)、AR2rep (SEQ ID NO: 61)、AR3rep (SEQ ID NO: 62)、AR4rep (SEQ ID NO: 63)、AR5rep (SEQ ID NO: 64) 及 AR6rep (SEQ ID NO: 65)。

以雌激素受體啟動子爲目標之可轉導人工轉錄因子

特定六聚鋅指蛋白由使用 ZiFit 軟體 v3.3 之所謂巴巴斯鋅指模組設置構成。爲了產生以雌激素受體爲目標之活性可轉導人工轉錄因子，使六聚鋅指蛋白以 ER_TS1 爲目標之 ZFP-ER1 (SEQ ID NO: 66)、以 ER_TS2 爲目標之 ZFP-ER2 (SEQ ID NO: 67)、及以 ER_TS3 爲目標之 ZFP-ER3 (SEQ ID NO: 68) 與蛋白轉導域 TAT 以及轉錄活化域 VP64 融合，得到人工轉錄因子 ER1akt (SEQ ID NO: 69)、ER2akt (SEQ ID NO: 70) 及 ER3akt (SEQ ID NO: 71)。爲了產生具有負調控活性之可轉導人工轉錄因子，使六聚鋅指蛋白 ZFP-ER1 至 ZFP-ER3 與蛋白轉導域 TAT 以及轉錄抑制域 SID 融合，得到人工轉錄因子 ER1rep (SEQ ID NO: 72)、ER2rep (SEQ ID NO: 74) 及 ER3rep (SEQ ID NO: 74)。

根據本發明之以糖皮質激素、雄激素或雌激素受體爲目標之人工轉錄

因子分別亦包含基於如 SEQ ID NO 39 至 41、48 至 53 及 66 至 68 所揭示之鋅指模組組成之鋅指蛋白，其中至多四個個別鋅指模組與具有替代性結合特徵之其他鋅指模組交換以調節人工轉錄因子與其目標序列之結合。

亦考慮含有五聚或六聚或七聚鋅指蛋白的本發明之人工轉錄因子，其中個別鋅指模組經交換以改善對各別核受體啟動子基因之目標位點的結合親和性或改變鋅指蛋白之免疫學特徵以改善可耐受性。

根據本發明之以核受體糖皮質激素、雄激素及或雌激素受體為目標之人工轉錄因子分別亦包含基於如 SEQ ID NO 39 至 41、48 至 53 及 66 至 68 所揭示之鋅指模組組成的鋅指蛋白，其中個別胺基酸經交換以便使潛在免疫原性減至最小，同時保留與所欲目標位點之結合親和性。

本發明之人工轉錄因子亦可能含有由基因本體論 GO:0001071 定義之蛋白質的其他轉錄活性蛋白質域，諸如 N 末端 KRAB、C 末端 KRAB、SID 及 ERD 域，較佳為 SID。所考慮之活化性蛋白質域為由基因本體論 go:0001071 定義之蛋白質之轉錄活性域，諸如 VP16 或 VP64（VP16 之四聚重複），較佳為 VP64。

此外，本發明之人工轉錄因子包含核定位序列（NLS）。所考慮之核定位序列為經由結合於由基因本體論 GO:0008139 定義之蛋白質來賦予核輸入之胺基酸基元，例如含有離胺酸殘基（K）、隨後離胺酸（K）或精胺酸殘基（R）、隨後任何胺基酸（X）、隨後離胺酸或精胺酸殘基之鹼性胺基酸叢集（K-K/R-X-K/R 一致序列，Chelsky D.等人, 1989 *Mol Cell Biol* 9, 2487-2492）或 SV40 NLS（SEQ ID NO: 37），其中 SV40 NLS 為較佳。

針對核受體基因之啟動子區域但無蛋白轉導域之人工轉錄因子亦為本

發明之主題。其為如上文所定義之本發明之人工轉錄因子的中間物。針對核受體基因之啟動子區域但無蛋白轉導域之該等人工轉錄因子的特定具體實例為針對雄激素受體基因啟動子之人工轉錄因子，及針對雌激素受體基因啟動子之人工轉錄因子，全部均無蛋白轉導域。

另外考慮用於藉由轉染或經由病毒載體（諸如（但不限於）基於疱疹病毒、腺病毒及腺相關病毒之載體）傳遞之核酸形式的本發明之人工轉錄因子的替代性傳遞方法。

本發明之人工轉錄因子的域可由短的可撓性連接子連接。短的可撓性連接子具有 2 至 8 個胺基酸，較佳為甘胺酸及絲胺酸。所考慮之特定連接子為 GGSGGS (SEQ ID NO: 76)。人工轉錄因子可進一步含有易於其偵測及加工之標記物。

在人工轉錄因子處理後評估糖皮質激素受體調節

就地塞米松 (dexamethasone) 處理後之轉錄誘導而言，將用糖皮質激素受體啟動子特異性負調控人工轉錄因子處理之海拉細胞 (HeLa cell) 與對照物處理之細胞進行比較。使用定量 RT-PCR 量測以基因 TSC22D3、IGFBP1 及 IRF8 為目標之糖皮質激素受體的表現量。與對照組細胞相比，在人工轉錄因子處理之細胞中此等糖皮質激素反應性基因之表現減少證明糖皮質激素受體特異性人工轉錄因子之調控活性。

在人工轉錄因子處理後評估雄激素受體調節

就睪固酮處理後之轉錄誘導而言，將用雄激素受體啟動子特異性負調控人工轉錄因子處理之表現雄激素受體之細胞與對照物處理之細胞進行比較。使用定量 RT-PCR 量測以基因 PSA、SPAK 及 TMPRSS2 為目標之雄激

素受體的表現量。與對照組細胞相比，在人工轉錄因子處理之細胞中此等雄激素反應性基因之表現減少證明雄激素受體特異性人工轉錄因子之調控活性。

在人工轉錄因子處理後評估雌激素受體調節

就雌二醇處理後之轉錄誘導而言，將用雌激素受體啟動子特異性負調控人工轉錄因子處理之表現雌激素受體之細胞與對照物處理之細胞進行比較。使用定量 RT-PCR 量測以基因 bcl-2、卵清蛋白、c-fos、膠原酶及催產素為目標之雌激素受體的表現量。與對照組細胞相比，在人工轉錄因子處理之細胞中此等雌二醇反應性基因之表現減少證明雌激素受體特異性人工轉錄因子之調控活性。

在螢光素酶報導分子分析中評估 AR4rep 活性

使用基於含有在雜交 CMV/AR_TS4 啟動子控制下之高斯椰屬螢光素酶及在組成性 CMV 啟動子控制下之分泌型鹼性磷酸酶的 HEK 293 FlpInTRex 細胞之報導分子細胞系來評估 AR4rep 之活性。如圖 5 中所示，用 AR4rep 處理該等細胞可引起與對照物處理之細胞相比螢光素酶活性減少。

聚乙二醇殘基之連接

認為將聚乙二醇殘基共價連接（聚乙二醇化）於本發明之人工轉錄因子可增加人工轉錄因子之可溶性，降低其腎清除率，且控制其免疫原性。考慮胺以及大小範圍為 1 至 40 千道爾頓 (kilodalton) 之硫醇活性聚乙二醇。使用硫醇活性聚乙二醇，達成人工轉錄因子之位點特異性聚乙二醇化。在本發明之人工轉錄因子中僅含有必要硫氫基之胺基酸為對於鋅配位必不可少的位於鋅指模組中之半胱胺酸殘基。此等硫氫基由於其鋅配位而不易用

於聚乙二醇化，因此本發明之人工轉錄因子中包涵一個或若干個半胱胺酸殘基提供自由硫氫基用於使用硫醇特異性聚乙二醇試劑之聚乙二醇化。

醫藥組成物

本發明亦關於包含如上文所定義之人工轉錄因子的醫藥組成物。所考慮之醫藥組成物為用於非經腸全身投藥（尤其靜脈內投藥）之組成物，用於吸入之組成物，用於向溫血動物（尤其人類）局部投藥（尤其眼部局部投藥（例如以滴眼劑形式），或玻璃體內、結膜下、眼旁（parabulbar）或眼球後投藥）之組成物。尤其較佳為滴眼劑及用於玻璃體內、結膜下、眼旁或眼球後投藥之組成物。組成物包含單獨活性成分或較佳以及醫藥學上可接受之載劑。另外考慮緩慢釋放調配物。活性成分之劑量視所治療之疾病及物種、其年齡、體重及個體病狀、個體藥物動力學資料及投藥方式而定。

另外考慮適用於經口傳遞之醫藥組成物，尤其為包含經適當囊封或以其他方式防止在內臟中降解之活性成分的組成物。舉例而言，該等醫藥組成物可含有膜滲透性增強劑、蛋白酶抑制劑，且由腸溶包衣包裹。

醫藥組成物包含約 1% 至約 95% 活性成分。單位劑型為例如安瓿、小瓶、吸入器、滴眼劑及其類似物。

本發明之醫藥組成物以自身已知方式製備，例如藉助於習知混合、溶解或凍乾過程。

較佳使用活性成分之溶液，且亦可在使用之前製備懸浮液或分散液，尤其為等張水性溶液、懸浮液或分散液，例如在凍乾組成物之情況下其包含單獨活性成分或以及載劑（例如甘露糖醇）。醫藥組成物可經滅菌及/或可包含賦形劑，例如防腐劑、穩定劑、潤濕劑及/或乳化劑、增溶劑、用於調

節滲透壓力之鹽及/或緩衝液且以自身已知之方式製備，例如藉助於習知溶解及凍乾過程。該等溶液或懸浮液可包含黏度增加劑，典型地為羧甲基纖維素鈉、羧甲基纖維素、葡聚糖、聚乙烯吡咯啉酮或明膠，或亦為增溶劑，例如 Tween 80™（聚氧化乙烯（20）山梨聚糖單油酸酯）。

於油中之懸浮液包含習知用於注射目的之植物油、合成油或半合成油作為油組分。關於此方面，可特別提及液體脂肪酸酯，其含有具有 8 至 22 個、尤其 12 至 22 個碳原子之長鏈脂肪酸作為酸組分。此等脂肪酸酯之醇組分具有最多 6 個碳原子且為單價或多價（例如單價、二價或三價）醇，尤其為乙二醇及甘油。作為脂肪酸酯之混合物，諸如棉籽油、杏仁油、橄欖油、蓖麻油、芝麻油、大豆油及花生油之植物油尤其適用。

可注射製劑之製造通常在無菌條件下進行，容器之填充（例如填充至安瓿或小瓶中）及密封亦如此。

對於非經腸投藥，水溶性形式之活性成分（例如水溶性鹽）之水性溶液或含有黏度增加物質（例如羧甲基纖維素鈉、山梨糖醇及/或葡聚糖）及（需要時）穩定劑之水性注射懸浮液尤其適合。活性成分，視情況以及賦形劑亦可呈凍乾物形式且可在非經腸投藥之前藉由添加適合之溶劑而製成溶液。

用於吸入之組成物可以氣溶膠形式、以噴霧、薄霧形式或以滴劑形式投予。氣溶膠由可用定劑量吸入器或霧化器傳遞之溶液或懸浮液製備，該定劑量吸入器或霧化器亦即為使用適合之推進劑（例如二氯二氟-甲烷、三氯氟甲烷、二氯四氟乙烷、二氧化碳或其他適合之氣體）以由患者吸入之霧化藥物之短脈衝形式將特定量之藥物傳遞至氣道或肺中的裝置。亦可提

供用於吸入之具有適合粉末基劑（諸如乳糖或澱粉）之粉末噴霧。

滴眼劑較佳為活性成分之等張水性溶液，其包含適合之藥劑以使得組成物與淚液等張（295-305 mOsm/l）。所考慮之藥劑為氯化鈉、檸檬酸、甘油、山梨糖醇、甘露糖醇、乙二醇、丙二醇、右旋糖及其類似物。此外，組成物包含緩衝劑，例如磷酸鹽緩衝液、磷酸鹽-檸檬酸鹽緩衝液或 Tris 緩衝液（參(羥甲基)-胺基甲烷）以便使 pH 值維持在 5 與 8 之間、較佳為 7.0 至 7.4。組成物可進一步含有抗微生物防腐劑，例如對羥苯甲酸酯、四級銨鹽（諸如氯化苄烷銨）、聚六亞甲基雙胍（polyhexamethylene biguanidine，PHMB）及其類似物。滴眼劑可進一步含有黃原膠以產生膠狀滴眼劑，及/或其他黏度增強劑，諸如玻尿酸、甲基纖維素、聚乙烯醇或聚乙烯吡咯啉酮。

人工轉錄因子在治療方法中之用途

此外，本發明係關於一種經組裝以便以如上文所述之核受體的啟動子區域為目標之人工轉錄因子，其用於影響細胞對核受體配位體之反應，用於降低或增加核受體含量，及用於治療由該等核受體調節之疾病。同樣，本發明係關於一種治療由核受體配位體調節之疾病的方法，其包含向有需要之患者投予治療有效量之針對核受體啟動子之人工轉錄因子。

由核受體配位體調節之疾病為例如腎上腺功能不全、腎上腺皮質功能不全、酒精中毒、阿茲海默氏病（Alzheimer's disease）、雄激素不敏感症候群、神經性厭食、主動脈瘤、主動脈瓣硬化、關節炎、哮喘、動脈粥樣硬化、注意力不足過動症、孤獨症、精子缺乏症、膽汁原發性肝硬化、躁鬱症、膀胱癌、骨癌、乳癌、心血管疾病、心血管心肌梗死、乳糜瀉、膽汁

鬱積、慢性腎衰竭及代謝症候群、肝硬化、齶裂、結腸直腸癌、先天性腎上腺發育不全、冠心病、隱睪、深靜脈血栓形成、癡呆、抑鬱症、糖尿病性視網膜病、乾眼病、子宮內膜異位、子宮內膜癌、增強 S 視錐症候群 (enhanced S-cone syndrome)、原發性高血壓、家族性部分脂肪代謝障礙、神經膠質母細胞瘤、糖皮質激素抗性、格雷夫氏症 (Graves' Disease)、高血清脂質含量、高阿樸 β 脂蛋白血症 (hyperapobetalipoproteinemia)、高脂血症、高血壓、高甘油三酯血症、低促性腺激素性腺機能減退 (hypogonadotropic hypogonadism)、尿道下裂、不育症、發炎性腸病、胰島素抗性、缺血性心臟病、肝皮脂腺病、肺癌、紅斑狼瘡、重度抑鬱症、男性乳癌、代謝血漿脂質含量、代謝症候群、偏頭痛、多發性硬化症、心肌梗塞、腎病症候群、非霍奇金氏淋巴瘤 (non-Hodgkin's lymphoma)、肥胖症、骨關節炎、骨質缺乏症、骨質疏鬆症、卵巢癌、帕金森氏病 (Parkinson's disease)、子癇前期、孕酮抗性、前列腺癌、假性醛固酮減少症、牛皮癬、精神病學精神分裂症、精神病、色素性視網膜炎-37、精神分裂症、硬化性膽管炎、性反轉、皮膚癌、肯尼迪脊髓延髓萎縮 (spinal and bulbar atrophy of Kennedy)、心肌梗塞易患性、牛皮癬易患性、睪丸癌、I 型糖尿病、II 類型糖尿病、子宮癌及眩暈。

同樣，本發明係關於一種治療由核受體配位體調節之疾病的方法，其包含向有需要之患者投予治療有效量之本發明之人工轉錄因子。詳言之，本發明係關於一種治療以下疾病之方法：腎上腺功能不全、腎上腺皮質功能不全、酒精中毒、阿茲海默氏病、雄激素不敏感症候群、神經性厭食、主動脈瘤、主動脈瓣硬化、關節炎、哮喘、動脈粥樣硬化、注意力不足過動症、孤獨症、精子缺乏症、膽汁原發性肝硬化、躁鬱症、膀胱癌、骨癌、

乳癌、心血管疾病、心血管心肌梗死、乳糜瀉、膽汁鬱積、慢性腎衰竭及代謝症候群、肝硬化、齶裂、結腸直腸癌、先天性腎上腺發育不全、冠心病、隱睪、深靜脈血栓形成、癡呆、抑鬱症、糖尿病性視網膜病、乾眼病、子宮內膜異位、子宮內膜癌、增強 S 視錐症候群、原發性高血壓、家族性部分脂肪代謝障礙、神經膠質母細胞瘤、糖皮質激素抗性、格雷夫氏症、高血清脂質含量、高阿撲 β 脂蛋白血症、高脂血症、高血壓、高甘油三酯血症、低促性腺激素性腺機能減退、尿道下裂、不育症、發炎性腸病、胰島素抗性、缺血性心臟病、肝皮脂腺病、肺癌、紅斑狼瘡、重度抑鬱症、男性乳癌、代謝血漿脂質含量、代謝症候群、偏頭痛、多發性硬化症、心肌梗塞、腎病症候群、非霍奇金氏淋巴瘤、肥胖症、骨關節炎、骨質缺乏症、骨質疏鬆症、卵巢癌、帕金森氏病、子癇前期、孕酮抗性、前列腺癌、假性醛固酮減少症、牛皮癬、精神病學精神分裂症、精神病、色素性視網膜炎-37、精神分裂症、硬化性膽管炎、性反轉、皮膚癌、肯尼迪脊髓延髓萎縮、心肌梗塞易患性、牛皮癬易患性、睪丸癌、I 型糖尿病、II 類型糖尿病、子宮癌及眩暈，該方法包含向有需要之患者投予有效量之本發明之人工轉錄因子。本發明之人工轉錄因子之有效量視所治療之疾病特定類型及物種、其年齡、體重及個體病狀、個體藥物動力學資料及投藥方式而定。對於投藥至眼睛中，每月玻璃體注射 0.5 至 1 mg 為較佳。對於全身施用，每月注射 10 mg/kg 為較佳。另外，將緩慢釋放沈澱物移植至眼睛玻璃體中亦為較佳。

此外，本發明係關於一種針對如上文所述之雄激素受體之人工轉錄因子，其用於影響細胞對雄激素受體配位體之反應，用於降低或增加雄激素

受體含量，及用於治療由雄激素受體配位體調節之疾病。

同樣，本發明係關於一種治療由雄激素受體配位體調節之疾病的方法，其包含向有需要之患者投予治療有效量之本發明之人工轉錄因子。所考慮之疾病為前列腺癌、男性乳癌、卵巢癌、結腸直腸癌、子宮內膜癌、睪丸癌、冠狀動脈病、I 型糖尿病、糖尿病性視網膜病、肥胖症、雄激素不敏感症候群、骨質疏鬆症、骨關節炎、II 型糖尿病、阿茲海默氏病、偏頭痛、注意力不足過動症、抑鬱症、精神分裂症、精子缺乏症、子宮內膜異位及肯尼迪脊髓延髓萎縮。詳言之，上調 AR 含量有益於治療乾眼病，而下調 AR 含量有益於治療 AR 阻斷非敏感性前列腺癌。本發明之人工轉錄因子之有效量視所治療之疾病特定類型及物種、其年齡、體重及個體病狀、個體藥物動力學資料及投藥方式而定。對於投藥至眼睛中，每月玻璃體注射 0.5 至 1 mg 為較佳。對於全身施用，每月注射 10 mg/kg 為較佳。另外，將緩慢釋放沈澱物移植至眼睛玻璃體中亦為較佳。

此外，本發明係關於一種針對如上文所述之雌激素受體之人工轉錄因子，其用於影響細胞對雌激素受體配位體之反應，用於降低或增加雌激素受體含量，及用於治療由雌激素受體配位體調節之疾病。

同樣，本發明係關於一種治療由雌激素受體配位體調節之疾病的方法，其包含向有需要之患者投予治療有效量之本發明之人工轉錄因子。所考慮之疾病為骨癌、乳癌、結腸直腸癌、子宮內膜癌、前列腺癌、子宮癌、酒精中毒、偏頭痛、主動脈瘤、心肌梗塞易患性、主動脈瓣硬化、心血管疾病、冠狀動脈病、高血壓、深靜脈血栓形成、格雷夫氏病、關節炎、多發性硬化症、肝硬化、B 型肝炎、慢性肝病、膽汁鬱積、尿道下裂、肥胖症、

骨關節炎、骨質缺乏症、骨質疏鬆症、阿茲海默氏病、帕金森氏病、偏頭痛、眩暈)、神經性厭食、注意力不足過動症、癡呆、抑鬱症、精神病、子宮內膜異位及不育症。詳言之，下調 ER 含量有益於治療激素依賴性乳癌。本發明之人工轉錄因子之有效量視所治療之疾病特定類型及物種、其年齡、體重及個體病狀、個體藥物動力學資料及投藥方式而定。對於投藥至眼睛中，每月玻璃體注射 0.5 至 1 mg 為較佳。對於全身施用，每月注射 10 mg/kg 為較佳。另外，將緩慢釋放沈澱物移植至眼睛玻璃體中亦為較佳。

人工轉錄因子在動物中之用途

此外，本發明係關於動物中所見之以核受體為目標之人工轉錄因子的用途，其用於治療藉由該等核受體之功能異常調節之疾病。較佳地，將人工轉錄因子直接應用於適於表面施用於有需要之動物之組成物中。

實施例

DNA 質體之選殖

對於所有選殖步驟，限制性核酸內切酶及 T4 DNA 連接酶購自 New England Biolabs。蝦鹼性磷酸酶 (Shrimp Alkaline Phosphatase, SAP) 來自 Promega。在所有標準 PCR 反應中應用高保真度鉑 Pfx DNA 聚合酶 (Invitrogen)。根據製造商之說明書，使用 NucleoSpin Gel 及 PCR Clean-up 套組、NucleoSpin Plasmid 套組或 NucleoBondXtra Midi Plus 套組 (Macherey-Nagel) 分離 DNA 片段及質體。寡聚核苷酸購自 Sigma-Aldrich。新產生之質體的所有相關 DNA 序列均藉由測序 (Microsynth) 來檢驗。

用於酵母單雜交之六聚鋅指蛋白文庫之選殖

根據 Gonzalez B.等人,2010, *Nat Protoc* 5, 791-810，經以下改良後，選殖

含有 GNN 及/或 CNN 及/或 ANN 結合鋅指 (ZF) 模組之選殖六聚鋅指蛋白文庫。合成編碼 GNN、CNN 及 ANN ZF 模組之 DNA 序列且分別插入 pUC57 (GenScript) 中，產生 pAN1049 (SEQ ID NO: 77)、pAN1073 (SEQ ID NO: 78) 及 pAN1670 (SEQ ID NO: 79)。鋅指蛋白 (ZFP) 文庫之逐步組裝在 pBluescript SK (+) 載體中進行。為了避免在每一個別選殖步驟期間插入多個 ZF 模組而產生非功能性蛋白質，pBluescript (及其含有 1 個 ZFP、2 個 ZFP 或 3 個 ZFP 之衍生產物) 且首先將 pAN1049、pAN1073 或 pAN1670 與一種限制酶一起培育，隨後用 SAP 處理。在添加第二種限制酶之前使用 NucleoSpin Gel 及 PCR Clean-up 套組移除酶。

藉由用 *XhoI*、SAP 及隨後 *SpeI* 處理 5 μ g pBluescript 進行 pBluescript-1ZFPL 之選殖。藉由將 10 μ g pAN1049 (釋放 16 個不同 GNN ZF 模組) 或 pAN1073 (釋放 15 個不同 CNN ZF 模組) 或 pAN1670 (釋放 15 個不同 ANN ZF 模組) 與 *SpeI*、SAP 及隨後 *XhoI* 一起培育來產生插入物。為產生 pBluescript-2ZFPL 及 pBluescript-3ZFPL，用 *AgeI* 切割 7 μ g pBluescript-1ZFPL 或 pBluescript-2ZFPL，去磷酸，且用 *SpeI* 切割。藉由分別將 *SpeI*、SAP 及隨後 *XmaI* 施用於 10 μ g pAN1049 或 pAN1073 或 pAN1670 來獲得插入物。藉由用 *AgeI*、SAP 及其後 *SpeI* 處理 14 μ g pBluescript-3ZFPL 以獲得經切割載體來進行 pBluescript-6ZFPL 之選殖。藉由與 *SpeI*、SAP 及隨後 *XmaI* 一起培育而自 20 μ g pBluescript-3ZFPL 釋放 3ZFPL 插入物。

在 RT(室溫)下以 20 μ l 總體積以 3:1 莫耳比之插入物:載體使用 200 ng 經切割載體、400 U T4 DNA 連接酶設置用於含有一個、兩個及三個 ZFP 之文庫的連接反應隔夜。六聚鋅指蛋白文庫之連接反應物包括 200 μ l 總體積

之 2000 ng pBluescript-3ZFPL、500 ng 3ZFPL 插入物、4000 U T4 DNA 連接酶，將其分成十份 20 μ l 且在 RT 下分開培育隔夜。藉由若干方法使連接反應物之部分轉型至大腸桿菌 (*Escherichia coli*) 中，此視每一文庫所需之純系數目而定。為產生 pBluescript-1ZFPL 及 pBluescript-2ZFPL，將 3 μ l 連接反應物直接用於大腸桿菌 NEB 5- α 之熱休克轉型。使用 NucleoSpin Gel 及 PCR Clean-up 套組純化 pBluescript-3ZFPL 之連接反應物之質體 DNA 且轉型至電穿孔勝任大腸桿菌 NEB 5- α 中（來自 EquiBio 之 EasyjecT Plus 電穿孔儀或來自 Eppendorf 之 Multiporator，2.5 kV 及 25 μ F，來自 Bio-Rad 之 2 mm 電穿孔小試管）。將 pBluescript-6ZFP 文庫之連接反應物施加於 NucleoSpin Gel 及 PCR Clean-up 套組且用 15 μ l 去離子水洗提 DNA。將約 60 ng 去鹽 DNA 與 50 μ l NEB 10- β 電穿孔勝任大腸桿菌 (New England Biolabs) 混合且如製造商所推薦使用 EasyjecT Plus 或 multiporator，2.5 kV、25 μ F 及 2 mm 電穿孔小試管進行電穿孔。對於每一文庫進行多次電穿孔且隨後直接混合細胞以增加文庫大小。熱休克轉型或電穿孔後，將 SOC 培養基施用於細菌且在 37 $^{\circ}$ C 及 250 rpm 下 1 小時培育後，使用 30 μ l SOC 培養物進行連續稀釋且接種於含有胺苄青黴素之 LB 板上。次日，測定所得文庫純系之總數。另外，每一文庫選擇十個純系以分離質體 DNA 且藉由限制酶消化來檢查檢查插入物之合併。對此等質體中至少三個進行測序以檢驗文庫之多樣性。將剩餘 SOC 培養物轉移至含有胺苄青黴素之 100 ml LB 培養基且在 37 $^{\circ}$ C 及 250 rpm 下培養隔夜。使用彼等細胞製備每一文庫之質體 Midi DNA。

對於酵母單雜交篩，將六聚鋅指蛋白文庫轉移至相容的獵物載體。出於該目的，藉由用 *Xho*I/*Eco*RI 切割載體且插入經黏接之寡聚核苷酸 OAN971

(TCGACAGGCCCCAGGC
GGCCCTCGAGGATATCATGATGACTAGTGGCCAGGCCGGCCC, SEQ ID NO:
80) 及 OAN972 (AATTGGGCCCGGCCTGGCC
ACTAGTCATCATGATATCCTCGAGGGCCGCCTGGGCCTG, SEQ ID NO: 81)
來調節 pGAD10 (Clontech) 之多個選殖位點。切割所得載體 pAN1025 (SEQ
ID NO: 82) 且去磷酸, 藉由 *XhoI/SpeI* 自 pBluescript-6ZFP 釋放 6ZFP 文庫插
入物。如上文所述對 pBluescript-6ZFP 文庫進行連接反應及電穿孔至 NEB
10- β 電穿孔勝任大腸桿菌中。

對於改良之酵母單雜交篩選, 亦將六聚鋅指文庫轉移至改良之獵物載
體 pAN1375 (SEQ ID NO: 83) 中。如下構築此獵物載體: 用 *ApaI/NarI* 切割
pRS315 (SEQ ID NO: 84) 且插入經黏接之 OAN1143
(CGCCGCATGCATTCATGCAGGC C, SEQ ID NO: 85) 及 OAN1144
(TGCATGAATGCATGCGG, SEQ ID NO: 86), 得到 pAN1373 (SEQ ID NO:
87)。將來自 pAN1025 之 *SphI* 插入物連接至用 *SphI* 切割之 pAN1373 中以獲
得 pAN1375。

對於進一步改良之酵母單雜交篩選, 亦將六聚鋅指文庫轉移至改良之
獵物載體 pAN1920 (SEQ ID NO: 88) 中。

對於甚至進一步改良之酵母單雜交篩選, 將六聚鋅指文庫插入獵物載
體 pAN1992 (SEQ ID NO: 89) 中。

用於酵母單雜交篩選之誘餌質體之選殖

對於每一誘餌質體, 選擇在中間含有 18 bp 潛在人工轉錄因子目標位點
之 60 bp 序列且包括 *NcoI* 位點以用於限制分析。設計寡聚核苷酸且以該方

式黏接以產生 5' *Hind*III 及 3' *Xho*I 位點，其允許直接連接至用 *Hind*III/*Xho*I 切割之 pAbAi (Clontech) 中。使用用 *Nco*I 消化並測序來證實誘餌質體之組裝。

酵母菌株及培養基

釀酒酵母 (*Saccharomyces cerevisiae*) Y1H Gold 購自 Clontech，YPD 培養基及 YPD 瓊脂購自 CarlRoth。合成營養缺陷 (SD) 培養基含有 20 g/l 葡萄糖、6.8 g/l $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 、9.7 g/l $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (全部來自 Carl Roth)、1.4 g/l 酵母合成營養缺陷培養基補充劑、6.7 g/l 酵母氮鹼、0.1 g/l L-色胺酸、0.1 g/l L-白胺酸、0.05 g/l L-腺嘌呤、0.05 g/l L-組胺酸、0.05 g/l 尿嘧啶 (全部來自 Sigma-Aldrich)。SD-U 培養基含有除尿嘧啶外之所有組分，製備無 L-白胺酸之 SD-L。SD 瓊脂板不含有磷酸鈉，但含有 16 g/l Bacto 瓊脂 (BD)。短梗黴素 A (Aureobasidin A, AbA) 購自 Clontech。

誘餌酵母菌株之製備

以 20 μ l 之總體積用 BstBI 使約 5 μ g 每一誘餌質體線性化且將反應混合物之一半直接用於釀酒酵母 Y1H Gold 之熱休克轉型。在轉型前一天使用酵母細胞接種 5 ml YPD 培養基且在 RT 下在滾筒上生長隔夜。以 1:20 用新鮮 YPD 培養基稀釋一毫升此預培養物且在 30°C、225 rpm 下培育 2-3 小時。因為每一轉型反應，藉由離心收穫 1 OD₆₀₀ 細胞，將酵母細胞用 1 ml 無菌水洗滌一次及用 1 ml TE/LiAc (10 mM Tris/HCl (pH 7.5)、1 mM EDTA、100 mM 乙酸鋰) 洗滌一次。最後，使酵母細胞再懸浮於 50 μ l TE/LiAc 中且與 50 μ g 來自鮭魚精巢之單股 DNA (Sigma-Aldrich)、10 μ l BstBI 線性化誘餌質體 (參見上文) 及 300 μ l PEG/TE/LiAc (10 mM Tris/HCl (pH 7.5)、1 mM EDTA、100

mM 乙酸鋰、50% (w/v) PEG 3350) 混合。在 RT 下將細胞及 DNA 在滾筒上培育 20 分鐘，隨後置於 42°C 水浴中 15 分鐘。最後，藉由離心作用收集酵母細胞，再懸浮於 100 μ l 無菌水中且展佈於 SD-U 瓊脂板上。在 30°C 下培育 3 天後，選自來自每一轉型反應之在 SD-U 上生長之八個純系以分析其對短梗黴素 A (AbA) 之敏感性。在 RT 下使預培養物在滾筒上生長隔夜。對於每一培養物，量測 OD₆₀₀ 且用無菌水調整為 OD₆₀₀ = 0.3。由此第一種稀釋液，用無菌水製備五種其他 1:10 稀釋步驟。對於每一純系，將來自每一稀釋步驟之 5 μ l 點樣於含有 SD-U、SD-U 100 ng/ml AbA、SD-U 150 ng/ml AbA 及 SD-U 200 ng/ml AbA 之瓊脂板上。在 30°C 下培育 3 天後，選擇在 SD-U 上生長良好且對 AbA 最敏感之三個純系用於進一步分析。根據製造商之說明書，藉由 Matchmaker Insert Check PCR Mix 1 (Clontech) 檢驗誘餌質體穩定整合至酵母基因組中。三個純系中之一者用於隨後 Y1H 篩。

用六聚鋅指蛋白文庫轉型誘餌酵母菌株

將約 500 μ l 酵母誘餌菌株預培養物為稀釋於 1 l YPD 培養基中且在 30°C 及 225 rpm 下培育直至 OD₆₀₀ = 1.6-2.0 (約 20 小時)。藉由以迴旋式轉子離心 (5 分鐘, 1500 g, 4°C) 收集細胞。根據 Benatuil L. 等人, 2010, *Protein Eng Des Sel* 23, 155-159 進行電穿孔勝任細胞之製備。對於每一轉型反應，將 400 μ l 電穿孔勝任誘餌酵母細胞與 1 μ g 編碼 6ZFP 文庫之獵物質體混合且在冰上培育 3 分鐘。將細胞-DNA 懸浮液轉移至預冷卻之 2 mm 電穿孔小試管中。進行多個電穿孔反應 (EasyjecT Plus 電穿孔儀或 Multiporator, 2.5 kV 及 25 μ F) 直至所有酵母細胞懸浮液均已轉型。電穿孔後，將酵母細胞轉移至 100 ml YPD:1 M 山梨糖醇之 1:1 混合物中且在 30°C 及 225 rpm 下培育 60 分鐘。

藉由離心收集細胞且再懸浮於 1-2 ml SD-L 培養基中。將 200 μ l 等分試樣展佈於含有 1000-4000 ng/ml AbA 之 15 cm SD-L 瓊脂板上。另外，使用 50 μ l 細胞懸液製備 1/100 及 1/1000 稀釋液且將 50 μ l 未稀釋及稀釋之細胞接種於 SD-L 上。所有培養板均在 30°C 下培育 3 天。自具有經稀釋轉型體之培養板計算所得純系之總數。雖然具有未稀釋細胞之 SD-L 培養板指示所有轉型體均生長，但若獵物 6ZFP 成功結合於其誘餌目標位點，則含有 AbA 之 SD-L 培養板僅產生群落形成。

編碼 6ZFP 之獵物質體的正相互作用及回收率之檢驗

對於初始分析，自含有最高 AbA 濃度之 SD-L 培養板上挑選四十個大小良好之群落且在具有 1000-4000 ng/ml AbA 之 SD-L 上將酵母細胞再劃兩次以獲得單一群落。對於每一純系，使用一個群落接種 5 ml SD-L 培養基且在 RT 下使細胞生長隔夜。次日，用無菌水調整為 $OD_{600} = 0.3$ ，製備五種其他 1/10 稀釋液且將 5 μ l 每一稀釋步驟點樣於 SD-L、SD-L 500 ng/ml AbA、1000 ng/ml AbA、SD-L 1500 ng/ml AbA、SD-L 2000 ng/ml AbA、SD-L 2500 ng/ml AbA、SD-L 3000 ng/ml AbA 及 SD-L 4000 ng/ml AbA 培養板上。將純系根據其在高 AbA 濃度下生長之能力進行分級。自生長最好之純系，使用 5 ml 初始 SD-L 預培養物來離心細胞且使其再懸浮於 100 μ l 水或殘餘培養基中。添加 50 U 溶壁酶（Sigma-Aldrich，L2524）後，在 37°C 及 300 rpm 下在水平震盪器上培育細胞若干小時。藉由添加 10 μ l 20%（w/v）SDS 溶液來溶解所產生之球芽，藉由渦旋有力地混合 1 分鐘且在 -20°C 下冷凍至少 1 小時。隨後，添加來自 NucleoSpin Plasmid 套組之 250 μ l A1 緩衝液及一刮刀尖端之玻璃珠（Sigma-Aldrich，G8772）且藉由渦旋有力地混合試管 1 分鐘。藉

由添加來自 NucleoSpin Plasmid 套組之 250 μ l A2 緩衝液且在 RT 下培育至少 15 分鐘，隨後繼續標準 NucleoSpin Plasmid 套組方案來進一步改良質體分離。用 30 μ l 洗提緩衝液洗提後，藉由熱休克轉型將 5 μ l 質體 DNA 轉型至大腸桿菌 DH5 α 中。自含有胺苄青黴素之 LB 培養板挑選兩個個別群落，分離質體且對文庫插入物進行測序。針對在 6ZFP 間每一目標位點之一致序列分析所得結果。

用於產生用於測試可轉導人工轉錄因子活性之穩定螢光素酶/分泌型鹼性磷酸酶報導細胞系之報導質體之選殖

爲了產生含有在雜交 CMV/人工轉錄因子目標位點啟動子控制下以及在組成性CMV 啟動子控制下之分泌型鹼性磷酸酶之高斯椰屬螢光素酶之報導分子構築體，用 *AflIII/SpeI* 將含有人工轉錄因子結合位點之 42 bp 選殖至 pAN1660 (SEQ ID NO: 90) 中。此等報導分子構築體含有用於穩定整合至含有 FlpIn 位點之細胞 (諸如 HEK 293 FlpIn TRex (Invitrogen) 細胞) 中之 FlpIn 位點。使用寡聚核苷酸 OAN1612 (SEQ ID NO: 91) 及 OAN1613 (SEQ ID NO: 92) 產生用於測試以 AR_TS4 爲目標之人工轉錄因子的該報導分子構築體。

用於哺乳動物轉染之人工轉錄因子之選殖

使用標準程序利用 *AgeI/XhoI* 將編碼多指鋅指蛋白之 DNA 片段選殖至用於在哺乳動物細胞中表現爲所關注鋅指陣列、SV40 NLS、3x 真菌抗原決定基標記及 N 末端 KRAB 域 (pAN1255-SEQ ID NO: 93)、C 末端 KRAB 域 (pAN1258-SEQ ID NO: 94)、SID 域 (pAN1257-SEQ ID NO: 95) 或 VP64 活化域 (pAN1510-SEQ ID NO: 96) 之間的融合蛋白之哺乳動物表現載體中。

如下產生用於產生穩定轉染之四環素誘導型細胞之質體：使用

EcoRV/AgeI 將編碼包含多指鋅指域、調控域 (N 末端 KRAB、C 末端 KRAB、SID 或 VP64) 及 SV40 NLS 之人工轉錄因子的 DNA 片段選殖至 pAN2071 (SEQ ID NO: 97) 中。此等人工轉錄因子表現質體可藉由用 AAVS1 Left TALEN 及 AAVS1 Right TALEN (GeneCopoeia) 共轉染而整合至人類基因組中之 AAVS1 基因座中。

細胞培養及轉染

在 5% CO₂、37°C 下使海拉細胞在補充有 4.5 g/l 葡萄糖、10% 熱失活胎牛血清、2 mM L-麩醯胺酸及 1 mM 丙酮酸鈉 (全部均來自 Sigma-Aldrich) 之杜科貝爾氏改良伊格爾培養基 (Dulbecco's Modified Eagle's Medium, DMEM) 中生長。對於螢光素酶報導分子分析, 將 7000 個海拉細胞/孔接種於 96 孔板中。次日, 根據製造商之說明書, 使用 Effectene 轉染試劑 (Qiagen) 進行共轉染。以比率 3:1 使用編碼人工轉錄因子及螢光素酶之質體 midi 製備物。在轉染後 6 小時及 24 小時由每孔 100 μ l 新鮮 DMEM 替換培養基。

Flp-In[™] T-Rex[™] 293 表現細胞系之產生及維持

穩定的四環素誘導型 Flp-In[™] T-Rex[™] 293 表現細胞系由 Flp 重組酶介導之整合產生。使用 Flp-In[™] T-Rex[™] Core 套組, 藉由轉染 pFRT/lacZeo 目標位點載體及 pcDNA6/TR 載體來產生 Flp-In[™] T-Rex[™] 宿主細胞系。爲了產生誘導型 293 表現細胞系, 經由 Flp 重組酶介導之 DNA 重組在 Flp-In[™] T-Rex[™] 宿主細胞系中之 FRT 位點處整合含有所關注基因之 pcDNA5/FRT/TO 表現載體。在含有 (DMEM; 10% Tet-FBS; 2 mM 麩醯胺酸; 15 μ g/ml 殺稻瘟菌素 (blastidone) 及 100 μ g/ml 潮黴素) 之選擇培養基中維持穩定的 Flp-In[™]

T-Rex™ 表現細胞系。爲了誘導基因表現，添加四環素至 1 μ g/mL 之最終濃度。

使用 TALEN 產生及維持穩定表現人工轉錄因子之細胞系

爲了產生穩定表現在四環素誘導型啓動子控制下之人工轉錄因子之細胞系，根據製造商之推薦，使用 Effectene (Qiagen，轉染試劑)，用含有所關注人工轉錄因子之表現構築體及 AAVS1 Left TALEN 及 AAVS1 Right TALEN (GeneCopoeia) 質體之基於 pAN2071 共轉染細胞。轉染後 8 小時，抽出生長培養基，用 PBS 洗滌細胞且添加新鮮生長培養基。轉染後 24 小時，使細胞以 1:10 比率在含有 Tet-經批准 FBS (無四環素 FBS，Takara) 而無抗生素之生長培養基中分裂。轉染後 48 小時，嘌呤黴素選擇以細胞類型特異性濃度開始且使細胞保持在選擇壓力下 7-10 天。混合穩定細胞群落且在選擇培養基中加以維持。

藉由定量 RT-PCR 測定基因表現量

根據製造商之說明書，使用 RNeasy Plus Mini 套組 (Qiagen，Hilden, Germany) 自細胞分離總 RNA。將冷凍之細胞集結粒再懸浮於含有 10 μ l/ml β -巯基乙醇之 RLT Plus Lysis 緩衝液中。使用 QIAshredder 離心柱均質化後，將總溶解產物轉移至 gDNA Eliminator 離心柱以消除基因組 DNA。添加一體積之 70%乙醇且將總溶解產物轉移至 RNeasy 離心柱。若干個洗滌步驟後，用最終容積爲 30 μ l 之無 RNA 酶 (RNase) 水洗提 RNA。將 RNA 儲存於 -80 °C 下直至進一步使用。根據製造商之說明書，使用高容量 cDNA 反轉錄套組 (Applied Biosystems，Branchburg, New Jersey, USA) 進行 cDNA 之合成。以含有 2 μ l 10 $\times$ 緩衝液、0.8 μ l 25 $\times$ dNTP 混合物、2 μ l 10 $\times$ RT 隨機引子、

1 μ l Multiscribe 反轉錄酶及 4.2 μ l H₂O 之 20 μ l 總反應體積進行 cDNA 合成。添加最終體積為 10 μ l 之 RNA 且在以下條件下進行反應：在 25°C 下 10 分鐘、隨後在 37°C 下 2 小時及最後一步在 85°C 下 5 分鐘。以含有 1 μ l 20 $\times$ TaqMan Gene Expression Master 混合物、10.0 μ l TaqMan[®] Universal PCR Master 混合物（兩者皆來自 Biosystems, Branchburg, New Jersey, USA）及 8 μ l H₂O 之 20 μ l 總反應體積進行定量 PCR。對於每一反應，添加 1 μ l cDNA。使用 ABI PRISM 7000 序列偵測系統(Applied Biosystems, Branchburg, New Jersey, USA)) 在以下條件下進行 qPCR：起始步驟為在 50°C 下 2 分鐘，隨後在 95°C 下第一次變性 10 分鐘及由在 95°C 下 15 秒及在 60°C 下 1 分鐘之 40 個循環組成之另一步驟。

用於細菌表現之人工轉錄因子的選殖

使用標準程序用 *EcoRV*/*NotI* 將編碼人工轉錄因子之 DNA 片段選殖至基於 pET41a+ (Novagen) 之細菌表現載體 pAN983 (SEQ ID NO: 98) 中以用於在大腸桿菌中表現為人工轉錄因子與 TAT 蛋白轉導域之間的 His₆ 標記融合蛋白。

在適合之大腸桿菌宿主細胞（諸如以 *GR*、*AR* 或 *ER* 為目標之 BL21 (DE3)) 中用於細菌性產生組織蛋白酶 B 敏感性可轉導人工轉錄因子之表現構築體為 pAN2343 (SEQ ID NO: 99)、pAN2344 (SEQ ID NO: 100)、pAN2345 (SEQ ID NO: 101)、pAN2346 (SEQ ID NO: 102) 及 pAN2347 (SEQ ID NO: 103)。

人工轉錄因子蛋白之產生

使用用於特定人工轉錄因子之表現質體轉型之大腸桿菌 BL21 (DE3) 在補充有 100 μ M ZnCl₂ 之 1 l LB 培養基中生長直至達到 0.8 與 1 之間的

OD₆₀₀，且誘導用 1mM IPTG 誘導兩小時。藉由離心收穫細菌，藉由音波處理來製備細菌溶解產物，且純化包涵體。為此，藉由離心收集（5000 g，4°C，15 分鐘）包涵體且在 20 ml 結合緩衝液（50 mM HEPES、500 mM NaCl、10 mM 咪唑；pH 7.5）中洗滌三次。在冰上在 30 ml 結合緩衝液 A（50 mM HEPES、500 mM NaCl、10 mM 咪唑、6 M GuHCl；pH 7.5）中溶解經純化之包涵體一小時。在 4°C 及 13'000 g 下離心溶解之包涵體 40 分鐘且經由 0.45 μ m PVDF 過濾器過濾。使用 His-Trap 管柱陷阱管柱在 Äktaprime FPLC（gehealthcare）上使用結合緩衝液 A 及洗提緩衝液 B（50 mM HEPES、500 mM NaCl、500 mM 咪唑、6 M GuHCl；pH 7.5）純化 His 標記之人工轉錄因子。混合含有經純化之人工轉錄因子的洗提份且在 4°C 下在含有 SID 域之人工轉錄因子的情況下針對緩衝液 S（50 mM Tris-HCl、500 mM NaCl、200 mM 精胺酸、100 μ M ZnCl₂、5 mM GSH、0.5 mM GSSG、50% 甘油；pH 7.5），或針對用於含有 KRAB 域之人工轉錄因子之緩衝液 K（50 mM Tris-HCl、300 mM NaCl、500 mM 精胺酸、100 μ M ZnCl₂、5 mM GSH、0.5 mM GSSG、50% 甘油；pH 8.5）透析隔夜。透析後，在 4°C 下以 14' 000 rpm 離心蛋白質樣品 30 分鐘且使用 0.22 μ m Millex-GV 過濾型吸管尖（Millipore）無菌過濾。對於含有 VP64 活化域之人工轉錄因子，根據製造商之推薦，使用 His-Bond Ni-NTA 樹脂（Novagen），由可溶性組分（結合緩衝液：50 mM NaPO₄（pH 7.5）、500 mM NaCl、10 mM 咪唑；洗提緩衝液：50 mM HEPES（pH 7.5）、500 mM NaCl、500 mM 咪唑）產生蛋白質。針對 VP64-緩衝液（550 mM NaCl（pH 7.4）、400 mM 精胺酸、100 μ M ZnCl₂）透析蛋白質。

蛋白質轉導

用 0.01 至 1 μ M 人工轉錄因子處理生長至約 80%融合之細胞或模擬處理 2 小時至 120 小時，其中在 37°C 下每 24 小時在 OptiMEM 或生長培養基中隨意添加人工轉錄因子。視情況，將 10-500 μ M ZnCl₂ 添加至生長培養基中。對於免疫螢光，用 PBS 洗滌細胞一次，經胰蛋白酶作用且接種於玻璃蓋片上以作進一步分析。

免疫螢光

用 4%多聚甲醛固定細胞，用 0.15% Triton X-100 處理，用 10% BSA 阻斷且與小鼠抗-HA 抗體（1:500，H9658，Sigma）或小鼠抗-真菌劑（1:500，M5546，Sigma）一起培育隔夜。用 PBS/1% BSA 洗滌樣品三次，且與偶合於 Alexa Fluor 546（1:1000，Invitrogen）之山羊抗-小鼠抗體一起培育，且使用 DAPI（1:1000 之 1 mg/ml，3 分鐘，Sigma）進行對比染色。使用螢光顯微術分析樣品。

組合之螢光素酶/SEAP 啟動子活性分析

爲了測試人工轉錄因子之活性，使用報導分子細胞系。此報導分子細胞系基於含有在雜交 CMV/人工轉錄因子目標位點啟動子控制下之高斯椰屬螢光素酶及在組成性 CMV 啟動子控制下之分泌型鹼性磷酸酶的 HEK 293 FlpInTRex 細胞。

將每孔 1×10^5 個報導分子細胞接種於 6 孔板中 24 小時，隨後進行蛋白質轉導。接種後 24 小時，自培養板吸出培養基且用 PBS 洗滌細胞一次。對於蛋白質處理，用 OptiMEM 將 AR4rep 稀釋至 1 μ M 之最終濃度，添加至細胞中且在保溫箱（37°C；5% CO₂）中培育 2 小時。在蛋白質轉導後，使細胞在正常生長培養基中生長 24 小時。將上清液轉移至 96 孔板，且在 2000 rpm

下離心 5 分鐘。爲了量測高斯椰屬螢光素酶，根據製造商之說明書使用 PierceTM高斯椰屬螢光素酶發熱分析套組（Thermo Scientific）。將工作溶液平衡至室溫且以 1:100 稀釋度添加腔腸素（coelenterazine）。將 20 μ l 細胞上清液轉譯至不透明 96 孔板中且添加 50 μ l 工作溶液。培育 10 分鐘後，使用 MicroLumatPlus（Berthold Technologies）以 1.0 秒之積分時間量測發光。爲了量測分泌型鹼性磷酸酶，根據製造商之說明書使用化學發光 SEAP 報導基因分析（Roche）。以 1:4 用稀釋緩衝液稀釋細胞上清液且在 65°C 下加熱失活 5 分鐘。將 50 μ L 加熱失活樣品轉移至不透明 96 孔板且添加 50 μ L 失活緩衝液。在室溫下培育 5 分鐘後，添加由含 AP 受質 1:20 之受質緩衝液組成之 50 μ L 受質試劑且在室溫在輕輕攪拌下下培育 10 分鐘。使用 MicroLumatPlus（Berthold Technologies）以 1.0 秒之積分時間量測發光。

【符號說明】

無

序列表

<110> 艾立歐夫塔公司
<120> 調控核受體之人工轉錄因子及其治療用途
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<212> DNA
<213> 智人

<400> 22
agcaaacgtt tacagagctc tggacaaaat tgagcgcta tgtgtacatg gcaagtgttt 60
ttagtgtttg tgtgtttacc tgcttgtctg ggtgattttg cttttgagag tctggatgag 120
aaatgcatgg ttaaaggcaa ttccagacag gaagaaaggc agagaagagg gtagaaatga 180
cctctgattc ttggggctga gggttcttag agcaaatggc acaatgccac gagggccgat 240

ctatccctat gacggaatct aaggtttcag caagtaatcg ctggcttggc catggcttgc 300
tccctagttt gtaggagact ctccactct cccatctgcg cgctcttata agtcttgaaa 360
agaacccctg gcagccagga gcaggtattc ctatcgtcct tttcctccct ccctcgccctc 420
caccctgttg gttttttaga ttgggccttg gaaccaaatt tggtagtgct tggcctccag 480
gaaatctgga gccctggcgc ctaaaccttg gtttaggaaa gcaggagcta ttcaggaagc 540
aggggtcctc cagggctaga gctagcctct cctgccctcg cccacgctgc gccagcactt 600
gtttctccaa agccactagg caggcgctag cgcgcggtga ggggagggga gaaaaggaaa 660
ggggagggga gggaaaagga ggtgggaagg caaggaggcc ggcccgggtg gggcgggacc 720
cgactcgcaa actgttgcat ttgctctcca cctccacgcg cccctccga gatcccgggg 780
agccagcttg ctgggagagc gggacggtcc ggagcaagcc cagaggcaga ggaggcgaca 840
gagggaaaaa gggccgagct agccgctcca gtgctgtaca ggagccgaag ggacgcacca 900
cgccagcccc agcccggctc cagcgacagc caacgcctct tgcagcgcgg cggcttcgaa 960
gccgcccc gccagctgcc tttcctcttc ggtgaagttt 1000

<210> 23
<211> 1000
<212> DNA
<213> 智人

<400> 23
tgcattttaa aaatctgtta gctggaccag accgacaatg taacataat gccaaagctt 60
tggttcgtga cctgaggta tgtttggtat gaaaagggtca cattttatat tcagttttct 120
gaagttttgg ttgcataacc aacctgttga aggcatgaac acccatgtgc gccctaacca 180
aaggtttttc tgaatcatcc ttcacatgag aattcctaata gggaccaagt acagtactgt 240
ggtccaacat aaacacacaa gtcaggctga gagaatctca gaaggttgtg gaagggtcta 300
tctactttgg gagcattttg cagaggaaga aactgaggtc ctggcagggt gcatttctct 360
gatggcaaaa tgcagctctt cctatatgta taccctgaat ctccgcccc tccccctcag 420
atgccccctg tcagttcccc cagctgctaa atatagctgt ctgtggcttg ctgcgtatgc 480
aaccgcacac cccattctat ctgccctatc tcggttacag ttagtctctc ccaggggtca 540
tcctatgtac acactacgta tttctagcca acgaggagg ggaatcaaac agaaagagag 600
acaaacagag atataatcga gtcctggcacg gggcacataa ggcagcacat tagagaaagc 660
cggccccctg atcctgtctt cgcgtttatt ttaagcccag tcttccctgg gccaccttta 720
gcagatcctc gtgcgcccc gccccctggc cgtgaaactc agcctctatc cagcagcgac 780
gacaagtaaa gtaaagttca ggggaagctgc tctttgggat cgctccaaat cgagtgtgc 840
ctggagtgat gtttaagcca atgtcagggc aaggcaacag tccctggccg tccctccagca 900
cctttgtaat gcataatgac tcgggagacc agtacttaaa gtggaggcc cgggagccca 960
ggagctggcg gagggcggtc gtcctgggac tgcacttgc 1000

<210> 24
<211> 289
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 24

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Cys Gln
20 25 30

Pro Met Lys Arg Leu Thr Leu Gly Asn Asp Ile Met Ala Ala Ala Val
35 40 45

Arg Met Asn Ile Gln Met Leu Leu Glu Ala Ala Asp Tyr Leu Glu Arg
50 55 60

Arg Glu Arg Glu Ala Glu His Gly Tyr Ala Ser Met Leu Pro Tyr Pro
65 70 75 80

Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys Pro Tyr Lys
85 90 95

Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu Val Arg
100 105 110

His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys
115 120 125

Gly Lys Ser Phe Ser Arg Asn Asp Ala Leu Thr Glu His Gln Arg Thr
130 135 140

His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe
145 150 155 160

Ser Gln Ser Gly Asn Leu Thr Glu His Gln Arg Thr His Thr Gly Glu
165 170 175

Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly
180 185 190

Ser Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys
195 200 205

Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly His Leu Val Arg
210 215 220

His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys
225 230 235 240

Gly Lys Ser Phe Ser Thr Ser Gly Asn Leu Val Arg His Gln Arg Thr
245 250 255

His Thr Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu

<210> 25
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 25

Pro Val Arg Arg Pro Arg Arg Arg Arg Arg Arg Lys
1 5 10

<210> 26
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 26

Thr His Arg Leu Pro Arg Arg Arg Arg Arg Arg Lys
1 5 10

<210> 27
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 27

Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5

<210> 28
<211> 16
<212> PRT
<213> 果蠅

<400> 28

Arg Gln Ile Leu Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
1 5 10 15

<210> 29
<211> 18
<212> DNA
<213> 智人

<400> 29
cgcgcgagg gcgtgggg 18

<210> 30
<211> 18
<212> DNA
<213> 智人

<400> 30
cggggagcgg ccgggggt 18

<210> 31
<211> 18
<212> DNA

<213>	智人	
<400>	31	
	gcctccaccc gctccccg	18
<210>	32	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	32	
	ctccagggct agagctag	18
<210>	33	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	33	
	ggcccgggtgg gggcgggga	18
<210>	34	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	34	
	agcgggacgg tccggagc	18
<210>	35	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	35	
	gcaggagcta ttcaggaa	18
<210>	36	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	36	
	tccagcagcg acgacaag	18
<210>	37	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	37	
	gtccctggcc gtcctcca	18
<210>	38	
<211>	18	
<212>	DNA	
<213>	智人	
<400>	38	
	gltggaggcc cgggagcc	18
<210>	39	
<211>	168	
<212>	PRT	
<213>	人工序列	
<220>		
<223>	合成構築體	

<400> 39

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
1 5 10 15
Ser Asp Lys Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30
Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Glu Leu
35 40 45
Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60
Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln
65 70 75 80
Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95
Ser Phe Ser Arg Ser Asp Asn Leu Val Arg His Gln Arg Thr His Thr
100 105 110
Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
115 120 125
Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140
Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser His Thr Gly His Leu
145 150 155 160
Leu Glu His Gln Arg Thr His Thr
165

<210> 40
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 40

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
1 5 10 15
Ser Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30
Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
35 40 45
Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60
Glu Cys Gly Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
145 150 155 160

Thr Glu His Gln Arg Thr His Thr
165

<210> 41
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 41

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
1 5 10 15

Pro Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu
35 40 45

Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly His Leu Val Arg His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
145 150 155 160

Thr Glu His Gln Arg Thr His Thr

165

<210> 42
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 42

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys
50 55 60

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Glu Leu Val Arg His
85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100 105 110

Lys Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln Arg Thr His
115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140

Arg Ser Asp Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Asp
165 170 175

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser His Thr Gly His Leu Leu Glu His
195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260 265 270
Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285
Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300
Ser Glu Glu Asp Leu
305

<210> 43
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 43

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15
Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30
Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45
Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly His
50 55 60
Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65 70 75 80
Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu Val Arg His
85 90 95
Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100 105 110
Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln Arg Thr His
115 120 125
Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140
Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160
Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
165 170 175
Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190
Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu Thr Glu His

195

200

205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg

210215220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala

225230235240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp

245250255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu

260265270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp

275280285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile

290295300

Ser Glu Glu Asp Leu

305

<210> 44

<211> 309

<212> PRT

<213> 人工序列

<220>

<223> 合成構築體

<400> 44

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln

151015

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp

202530

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys

354045

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His

505560

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys

65707580

Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu Glu Arg His

859095

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly

100105110

Lys Ser Phe Ser Thr Ser Gly His Leu Val Arg His Gln Arg Thr His

115120125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser

130135140

Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
165 170 175

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu Thr Glu His
195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260 265 270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300

Ser Glu Glu Asp Leu
305

<210> 45
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 45

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg

85 90 95

Ser Asp Lys Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Glu Leu
115 120 125

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Arg Ser Asp Asn Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
195 200 205

Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser His Thr Gly His Leu
225 230 235 240

Leu Glu His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 46
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 46

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
85 90 95

Ser Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
115 120 125

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
225 230 235 240

Thr Glu His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 47
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 47

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala

35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
85 90 95

Pro Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu
115 120 125

Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly His Leu Val Arg His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Arg Ser Asp Asp Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu
225 230 235 240

Thr Glu His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 48
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 48

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
1 5 10 15

Ser Asp Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Ala Leu
35 40 45

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Gln Asn Ser Thr Leu Thr Glu His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
115 120 125

Ser Gly Glu Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Asn Ser Thr Leu
145 150 155 160

Thr Glu His Gln Arg Thr His Thr
165

- <210> 49
- <211> 168
- <212> PRT
- <213> 人工序列
- <220>
- <223> 合成構築體
- <400> 49

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
1 5 10 15

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Asp Leu
35 40 45

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu Val Arg His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Arg Ser Asp Glu Leu Val Arg His Gln Arg Thr His Thr

100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
115 120 125

Asn Asp Thr Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu
145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 50
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 50

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
1 5 10 15

Ser Gly Glu Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Ser Lys Lys His Leu
35 40 45

Ala Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Asp Pro Gly Asn Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
115 120 125

Asn Asp Thr Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu
145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 51
<211> 168
<212> PRT

<213> 人工序列

<220>

<223> 合成構築體

<400> 51

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
1 5 10 15

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
35 40 45

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Thr Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu
145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 52

<211> 168

<212> PRT

<213> 人工序列

<220>

<223> 合成構築體

<400> 52

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
1 5 10 15

Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
35 40 45

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Thr Ser Gly Ser Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu
145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 53
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 53

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
1 5 10 15

Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
35 40 45

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Gln Ser Gly Asp Leu Arg Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu

145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 54
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 54

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Asn
50 55 60

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Ala Leu Thr Glu His
85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100 105 110

Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln Arg Thr His
115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140

Gln Asn Ser Thr Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu
165 170 175

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Gln Asn Ser Thr Leu Thr Glu His
195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260 265 270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300

Ser Glu Glu Asp Leu
305

<210> 55
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 55

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
50 55 60

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Asp Leu Val Arg His
85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100 105 110

Lys Ser Phe Ser Arg Ser Asp Lys Leu Val Arg His Gln Arg Thr His
115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140

Arg Ser Asp Glu Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Thr
165 170 175

Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys

180

185

190

Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu Val Arg His
195200205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210215220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225230235240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245250255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260265270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275280285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290295300

Ser Glu Glu Asp Leu
305

<210> 56
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 56

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
151015

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
202530

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
354045

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu
505560

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65707580

Pro Glu Cys Gly Lys Ser Phe Ser Ser Lys Lys His Leu Ala Glu His
859095

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100105110

Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln Arg Thr His
115120125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140

Asp Pro Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Thr
165 170 175

Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu Val Arg His
195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260 265 270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300

Ser Glu Glu Asp Leu
305

<210> 57
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 57

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
50 55 60

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys

65707580

Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu Thr Glu His
859095

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100105110

Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln Arg Thr His
115120125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130135140

Thr Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145150155160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
165170175

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180185190

Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu Val Arg His
195200205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210215220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225230235240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245250255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260265270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275280285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290295300

Ser Glu Glu Asp Leu
305

<210> 58
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 58

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
151015

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
 20 25 30
 Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
 35 40 45
 Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Asn
 50 55 60
 Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 65 70 75 80
 Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu Thr Glu His
 85 90 95
 Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
 100 105 110
 Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln Arg Thr His
 115 120 125
 Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
 130 135 140
 Thr Ser Gly Ser Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
 145 150 155 160
 Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
 165 170 175
 Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 180 185 190
 Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu Val Arg His
 195 200 205
 Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
 210 215 220
 Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
 225 230 235 240
 Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
 245 250 255
 Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
 260 265 270
 Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
 275 280 285
 Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
 290 295 300
 Ser Glu Glu Asp Leu
 305

<210> 59
 <211> 309
 <212> PRT
 <213> 人工序列

<220>
 <223> 合成構築體

<400> 59

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
 1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
 20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
 35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Asn
 50 55 60

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu Thr Glu His
 85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
 100 105 110

Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln Arg Thr His
 115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
 130 135 140

Gln Ser Gly Asp Leu Arg Arg His Gln Arg Thr His Thr Gly Glu Lys
 145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
 165 170 175

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu Val Arg His
 195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
 210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
 225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
 245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu

260265270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275280285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290295300

Ser Glu Glu Asp Leu
305

<210> 60
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 60

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
151015

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
202530

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
354045

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
505560

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65707580

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
859095

Ser Asp Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100105110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Ala Leu
115120125

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130135140

Glu Cys Gly Lys Ser Phe Ser Asp Cys Arg Asp Leu Ala Arg His Gln
145150155160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165170175

Ser Phe Ser Gln Asn Ser Thr Leu Thr Glu His Gln Arg Thr His Thr
180185190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
195200205

Ser Gly Glu Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Asn Ser Thr Leu
225 230 235 240

Thr Glu His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 61
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 61

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
85 90 95

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Asp Leu
115 120 125

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Arg Ser Asp Lys Leu Val Arg His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Arg Ser Asp Glu Leu Val Arg His Gln Arg Thr His Thr

180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
195 200 205

Asn Asp Thr Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly His Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 62
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 62

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
85 90 95

Ser Gly Glu Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Ser Lys Lys His Leu
115 120 125

Ala Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Asp Pro Gly Asn Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg
195 200 205

Asn Asp Thr Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 63
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 63

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
85 90 95

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
115 120 125

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro

130

135

140

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Thr Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 64
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 64

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
85 90 95

Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
115 120 125

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Thr Ser Gly Ser Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 65
<211> 279
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 65

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr

85 90 95

Ser Gly Asn Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Arg Ala Asp Asn Leu
115 120 125

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser His Lys Asn Ala Leu Gln Asn His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Gln Ser Gly Asp Leu Arg Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 66
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 66

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
1 5 10 15

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu
35 40 45

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu Val Arg His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Asp Pro Gly Ala Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
115 120 125

Pro Gly Ala Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Thr Gly Ala Leu
145 150 155 160

Thr Glu His Gln Arg Thr His Thr
165

<210> 67
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 67

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
1 5 10 15

Ser His Ser Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Lys Asn Ser Leu
35 40 45

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
115 120 125

Lys Asn Ser Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu

145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 68
<211> 168
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 68

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
1 5 10 15

Cys Arg Asp Leu Ala Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
20 25 30

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu
35 40 45

Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
50 55 60

Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Thr Leu Thr Glu His Gln
65 70 75 80

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
85 90 95

Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr
100 105 110

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
115 120 125

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
130 135 140

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Ser Leu
145 150 155 160

Val Arg His Gln Arg Thr His Thr
165

<210> 69
<211> 309
<212> PRT
<213> 人工序列

<220>
<223> 合成構築體

<400> 69

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp

202530

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
354045

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
505560

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65707580

Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu Val Arg His
859095

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100105110

Lys Ser Phe Ser Thr Ser Gly Glu Leu Val Arg His Gln Arg Thr His
115120125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130135140

Asp Pro Gly Ala Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145150155160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala
165170175

Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180185190

Pro Glu Cys Gly Lys Ser Phe Ser Thr Thr Gly Ala Leu Thr Glu His
195200205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
210215220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225230235240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245250255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260265270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275280285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290295300

Ser Glu Glu Asp Leu
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<211> 309
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 <223> 合成構築體

<400> 70

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
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Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
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Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
 35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser His Ser
 50 55 60

Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Thr Lys Asn Ser Leu Thr Glu His
 85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
 100 105 110

Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln Arg Thr His
 115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
 130 135 140

Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
 145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Lys Asn Ser
 165 170 175

Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
 180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu Val Arg His
 195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
 210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
 225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
 245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
 260 265 270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300

Ser Glu Glu Asp Leu
305

<210> 71
<211> 309
<212> PRT
<213> 人工序列

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<223> 合成構築體

<400> 71

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
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Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro Gly Glu Lys
35 40 45

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Cys Arg Asp
50 55 60

Leu Ala Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
65 70 75 80

Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu Glu Arg His
85 90 95

Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly
100 105 110

Lys Ser Phe Ser Arg Asn Asp Thr Leu Thr Glu His Gln Arg Thr His
115 120 125

Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser
130 135 140

Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys
145 150 155 160

Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His
165 170 175

Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys
180 185 190

Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Ser Leu Val Arg His
195 200 205

Gln Arg Thr His Thr Gly Gly Gly Ser Gly Gly Ser Glu Phe Gly Arg
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210 215 220

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala
225 230 235 240

Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp
245 250 255

Phe Asp Leu Asp Met Leu Gly Ser Asp Ala Leu Asp Asp Phe Asp Leu
260 265 270

Asp Met Leu Ile Asn Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp
275 280 285

Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys Leu Ile
290 295 300

Ser Glu Glu Asp Leu
305

<210> 72
<211> 279
<212> PRT
<213> 人工序列

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<400> 72

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
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Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
85 90 95

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu
115 120 125

Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Glu Leu Val Arg His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Asp Pro Gly Ala Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
195 200 205

Pro Gly Ala Leu Val Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Thr Gly Ala Leu
225 230 235 240

Thr Glu His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 73
<211> 279
<212> PRT
<213> 人工序列

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<400> 73

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
85 90 95

Ser His Ser Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Lys Asn Ser Leu
115 120 125

Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro

130 135 140

Glu Cys Gly Lys Ser Phe Ser Ser Arg Arg Thr Cys Arg Ala His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr
195 200 205

Lys Asn Ser Leu Thr Glu His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp Pro Gly Ala Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 74
<211> 279
<212> PRT
<213> 人工序列

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<400> 74

Met His His His His His His Gly Tyr Gly Arg Lys Lys Arg Arg Gln
1 5 10 15

Arg Arg Arg Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Pro Trp Asp
20 25 30

Ile Met Ala Ala Ala Val Arg Met Asn Ile Gln Met Leu Leu Glu Ala
35 40 45

Ala Asp Tyr Leu Glu Arg Arg Glu Arg Glu Ala Glu His Gly Tyr Ala
50 55 60

Ser Met Leu Pro Tyr Pro Lys Lys Lys Arg Lys Val Gly Leu Glu Pro
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Asp
85 90 95

Cys Arg Asp Leu Ala Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
100 105 110

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln Arg Ala His Leu
115 120 125

Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro
130 135 140

Glu Cys Gly Lys Ser Phe Ser Arg Asn Asp Thr Leu Thr Glu His Gln
145 150 155 160

Arg Thr His Thr Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys
165 170 175

Ser Phe Ser Asp Pro Gly His Leu Val Arg His Gln Arg Thr His Thr
180 185 190

Gly Glu Lys Pro Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Gln
195 200 205

Arg Ala His Leu Glu Arg His Gln Arg Thr His Thr Gly Glu Lys Pro
210 215 220

Tyr Lys Cys Pro Glu Cys Gly Lys Ser Phe Ser Thr Ser Gly Ser Leu
225 230 235 240

Val Arg His Gln Arg Thr His Thr Gly Glu Gln Lys Leu Ile Ser Glu
245 250 255

Glu Asp Leu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Glu Gln Lys
260 265 270

Leu Ile Ser Glu Glu Asp Leu
275

<210> 75
<211> 7
<212> PRT
<213> 猿猴病毒40

<400> 75

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 76
<211> 6
<212> PRT
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<223> 合成構築體

<400> 76

Gly Gly Ser Gly Gly Ser
1 5

<210> 77
<211> 4513
<212> DNA
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<400> 77

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aggccctttc gtc 4513

<210> 78
<211> 4442
<212> DNA
<213> 人工序列

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cagaatgcgg aaagagttc tccagaagtg acaaattagt aagacaccag agaaccata	5160
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gaagtgcga tttagtaaga catcagcgca cgcacaccgg ggagaaaccc tataagtgtc	5520
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申請專利範圍

1. 一種人工轉錄因子，其包含與抑制性或活化性蛋白質域、核定位序列及蛋白轉導域融合之特異性以核受體基因之啓動子區域爲目標之多指 (polydactyl) 鋅指蛋白。
2. 如申請專利範圍第 1 項之人工轉錄因子，其中該核受體基因之啓動子區域爲雄激素受體啓動子。
3. 如申請專利範圍第 1 項之人工轉錄因子，其中該核受體基因之啓動子區域爲雌激素受體啓動子。
4. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其包含六聚鋅指蛋白。
5. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其中該鋅指蛋白與抑制性蛋白質域融合。
6. 如申請專利範圍第 5 項之人工轉錄因子，其中該抑制性蛋白質域爲 SEQ ID NO: 1 之 N 末端 KRAB、SEQ ID NO: 2 之 C 末端 KRAB、SEQ ID NO: 3 之 SID 或 SEQ ID NO: 4 之 ERD。
7. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其中該鋅指蛋白與活化性蛋白質域融合。
8. 如申請專利範圍第 7 項之人工轉錄因子，其中該活化性蛋白質域爲 SEQ ID NO: 5 之 VP16、SEQ ID NO: 6 之 VP64、SEQ ID NO: 7 之 CJ7、SEQ ID NO: 8 之 p65TA1、SEQ ID NO: 9 之 SAD、SEQ ID NO: 10 之 NF-1、SEQ ID NO: 11 之 AP-2、SEQ ID NO: 12 之 SP1-A、SEQ ID NO: 13 之 SP1-B、SEQ ID NO: 14 之 Oct-1、SEQ ID NO: 15 之 Oct-2、SEQ ID NO: 16 之 Oct2-5x、

SEQ ID NO: 17 之 MTF-1、SEQ ID NO: 18 之 BTEB-2 或 SEQ ID NO: 19 之 LKLF。

9. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其中該核定位序列為含有 K-K/R-X-K/R 一致序列之鹼性胺基酸叢集或 SEQ ID NO: 75 之 SV40 NLS。
10. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其中該蛋白轉導域為 SEQ ID NO: 20 之 HIV 衍生 TAT 肽、SEQ ID NO: 25 之合成肽 mT02、SEQ ID NO: 26 之合成肽 mT03、SEQ ID NO: 27 之 R9 肽或 SEQ ID NO: 28 之 ANTP 域。
11. 如申請專利範圍第 1 項之人工轉錄因子，其包含選自由 SEQ ID NO: 39 至 41、48 至 53 及 66 至 68 組成之群的蛋白質序列之鋅指蛋白。
12. 一種人工轉錄因子，其包含與抑制性或活化性蛋白質域及核定位序列融合之特異性以核受體基因之啟動子區域為目標之多指鋅指蛋白。
13. 如申請專利範圍第 1 項至第 3 項中任一項之人工轉錄因子，其進一步包含聚乙二醇殘基。
14. 一種醫藥組成物，其包含如申請專利範圍第 1 項至第 13 項中任一項之人工轉錄因子。
15. 一種用於產生如申請專利範圍第 1 項至第 12 項中任一項之人工轉錄因子之大腸桿菌 (*E. coli*) 宿主細胞，其含有 SEQ ID NO: 99 至 103 之表現構築體。
16. 一種用於調節細胞對核受體之配位體的反應之如申請專利範圍第 1 項至第 13 項之人工轉錄因子。

18. 一種如申請專利範圍第 2 項或第 4 項至第 13 項中任一項之人工轉錄因子的用途，其係用於製造用於治療由睪固酮調節且選自由癌症、冠狀動脈病、肥胖症、糖尿病、精神分裂症、抑鬱症及注意力不足過動症組成之群的疾病之醫藥品，其中該核受體為雄激素受體。
19. 一種如申請專利範圍第 3 項至第 13 項中任一項之人工轉錄因子的用途，其係用於製造用於治療由雌激素調節且選自由癌症、心血管疾病、骨質疏鬆症及情感障礙組成之群的疾病之醫藥品，其中該核受體為雌激素受體。
20. 一種如申請專利範圍第 1 項或第 4 項至第 13 項中任一項之人工轉錄因子的用途，其係用於製造用於治療由糖皮質激素調節且選自由發炎性過程、糖尿病、肥胖症、冠狀動脈病、哮喘、乳糜瀉及紅斑狼瘡組成之群的疾病之醫藥品，其中該核受體為糖皮質激素受體。

圖式

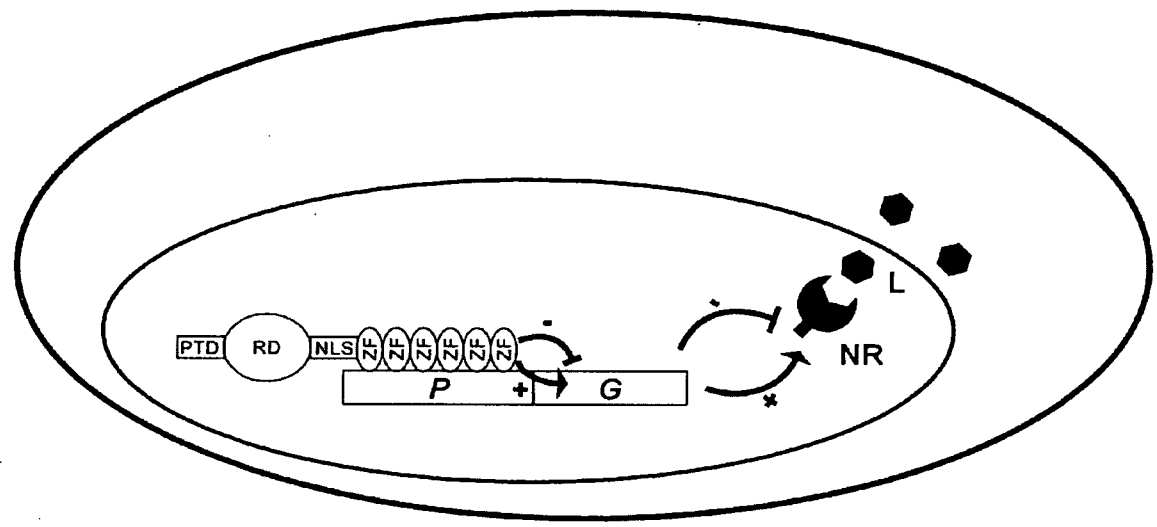


圖1

1	CGACTCCCCCGGGCCCAAAGTACGTATGCGCCGACCCCCGCTATCCCGTCCCTTCCCTG	60
61	AAGCCTCCCCAGAGGGCGTGTCAAGCCGCCCCGCCCCGAGCGCGGCCGAGACGCTGCGGC	120
121	ACCGTTTCCGTGCAACCCCGTAGCCCCCTTTCGAAGTGACACACTTCACGCAACTCGGCCC	180
181	GGCGGCGGGCGGCGGGGCCACTCACGCAGCTCAGCCGCGGGAGGCGCCCCGGCTCTTGTG	240
241	GCCCCGCCGCTGTCACCCGCAGGGGCACTGGCGGCGCTTGCCGCCAAGGGGCAGAGCGAG	300
301	CTCCCGAGTGGGTCTGGAGCCGCGGAGCTGGGCGGGGGCGGGGAAGGAGGTAGCGAGAAAA	360
361	GAAACTGGAGAAACTCGGTGGCCCTCTTAACGCCGCCCCAGAGAGACCAGGTCGGCCCCC	420
421	GCCGCTGCCGCCGCCACCCTTTTTCTGGGGAGTTGGGGGCGGGGGCGAAGCGCGGCGC	480
481	ACCGGGCGGGGCGGCCACGCCAGGGGACGCGGGCGTGCAGGCGCCGTCGGGGCCGGGGTG	540
541	GCGGGGCCCCGCGCGGAGGGCGTGGGGGACGGGACCGCGGGCGCCCCCTGCAGTTGCCAAGC	600
601	GTCACCAACAGGTTGCATCGTTCCCCGCGGCCGCCGCGCGGCCCTCGGGCGGGGAGCGG	660
661	<u>CCGGGGGTGGAGTGGGAGCGCGTGTGTGCGAGTGTGTGCGCGCCGTGGCGCCGCCTCCAC</u>	720
721	<u>CCGCTCCCCGCTCGGTCCCGCTCGCTCGCCAGGCCGGGCTGCCCTTTCGCGTGTCCGCG</u>	780
781	CTCTCTTCCCTCCGCCCGCCCTCCTCCATTTTGCGAGCTCGTGTCTGTGACGGGAGCCC	840
841	GAGTCACCGCCTGCCCGTCGGGGACGGATTCTGTGGGTGGAAGGAGACGCCGCAGCCGGA	900
901	GCGGCCGAAGCAGCTGGGACCGGGACGGGGCACGCGCGCCCCGGAACCTCGACCCGCGGAG	960
961	CCCGGCGCGGGGCGGAGGGCTGGCTTGTGAGCTGGGCAAT	1000

圖2

1	AGCAAACGTTTACAGAGCTCTGGACAAAATTGAGCGCCTATGTGTACATGGCAAGTGTTT	60
61	TTAGTGTTTGTGTGTTTACCTGCTTGTCTGGGTGATTTTGCCTTTGAGAGTCTGGATGAG	120
121	AAATGCATGGTTAAAGGCAATTCAGACAGGAAGAAAGGCAGAGAAGAGGGTAGAAATGA	180
181	CCTCTGATTCTTGGGGCTGAGGGTTCCTAGAGCAAATGGCACAAATGCCACGAGGCCCCGAT	240
241	CTATCCCTATGACGGAATCTAAGGTTTCAGCAAGTATCTGCTGGCTTGGTCATGGCTTGC	300
301	TCCTCAGTTTGTAGGAGACTCTCCCACTCTCCCATCTGCGCGCTCTTATCAGTCCTGAAA	360
361	AGAACCCCTGGCAGCCAGGAGCAGGTATTCCTATCGTCCTTTTCCTCCCTCCCTCGCCTC	420
421	CACCCTGTTGGTTTTTTTAGATTGGGCTTTGGAACCAAATTTGGTGAGTGCTGGCCTCCAG	480
481	GAAATCTGGAGCCCTGGCGCCTAAACCTTGGTTTTAGGAAAGCAGGAGCTATTCAGGAAGC	540
541	AGGGGTCCTCCAGGGCTAGAGCTAGCCTCTCCTGCCCTCGCCCACGCTGCGCCAGCACTT	600
601	GTTTCTCCAAAGCCACTAGGCAGGCGTTAGCGCGCGGTGAGGGGAGGGGAGAAAAGGAAA	660
661	GGGGAGGGGAGGGAAAAGGAGGTGGGAAGGCAAGGAGGCCGGCCCCGGTGGGGGCGGGACC	720
721	CGACTCGCAAAGTGTGCAATTTGCTCTCCACCTCCCAGCGCCCCCTCCGAGATCCCCGGG	780
781	AGCCAGCTTGCTGGGAGAGCGGGACGGTCCGGAGCAAGCCCAGAGGCAGAGGAGGCGACA	840
841	GAGGGAAAAAGGGCCGAGCTAGCCGCTCCAGTGCTGTACAGGAGCCGAAGGGACGCACCA	900
901	CGCCAGCCCCAGCCCGGCTCCAGCGACAGCCAACGCCTCTTGACGCGCGGCGGCTTCGAA	960
961	GCCGCCGCCCGGAGCTGCCCTTTCCTCTTCGGTGAAGTTT	1000

圖3

1	TGCATTTTAAAAATCTGTTAGCTGGACCAGACCGACAATGTAACATAATTGCCAAAGCTT	60
61	TGGTTCGTGACCTGAGGTTATGTTGGTATGAAAAGGTCACATTTTATATTCAGTTTTCT	120
121	GAAGTTTTGGTTGCATAACCAACCTGTGGAAGGCATGAACACCCATGTGCGCCCTAACCA	180
181	AAGGTTTTTCTGAATCATCCTTCACATGAGAATTCCTAATGGGACCAAGTACAGTACTGT	240
241	GGTCCAACATAAACACACAAGTCAGGCTGAGAGAATCTCAGAAGGTTGTGGAAGGGTCTA	300
301	TCTACTTTGGGAGCATTTTGCAGAGGAAGAACTGAGGTCCTGGCAGGTTGCATTCTCCT	360
361	GATGGCAAAATGCAGCTCTTCCTATATGTATACCCTGAATCTCCGCCCCCTTCCCCTCAG	420
421	ATGCCCCCTGTCTAGTTCCCCCAGCTGCTAAATATAGCTGTCTGTGGCTGGCTGCGTATGC	480
481	AACCGCACACCCCATTCTATCTGCCCTATCTCGGTTACAGTGTAGTCCTCCCCAGGGTCA	540
541	TCCTATGTACACACTACGTATTTCTAGCCAACGAGGAGGGGGAATCAAACAGAAAGAGAG	600
601	ACAAACAGAGATATATCGGAGTCTGGCACGGGGCACATAAGGCAGCACATTAGAGAAAGC	660
661	CGGCCCCCTGGATCCGTCTTTCGCGTTTATTTTAAGCCCAGTCTTCCCTGGGCCACCTTTA	720
721	GCAGATCCTCGTGCGCCCCCGCCCCCTGGCCGTGAAACTCAGCCTCTATCCAGCAGCGAC	780
781	<u>GACAAGTAAAGTAAAGTTCAGGGAAGCTGCTCTTTGGGATCGCTCCAAATCGAGTTGTGC</u>	840
841	CTGGAGTGATGTTTAAGCCAATGTCAGGGCAAGGCAACAGTCCCTGGCCGTCCTCCAGCA	900
901	CCTTTGTAATGCATATGAGCTCGGGAGACCAGTACTTAAAGT <u>TGGAGGCCCGGGAGCCCA</u>	960
961	GGAGCTGGCGGAGGGCGTTCGTCCTGGGACTGCACTTGCT	1000

圖4

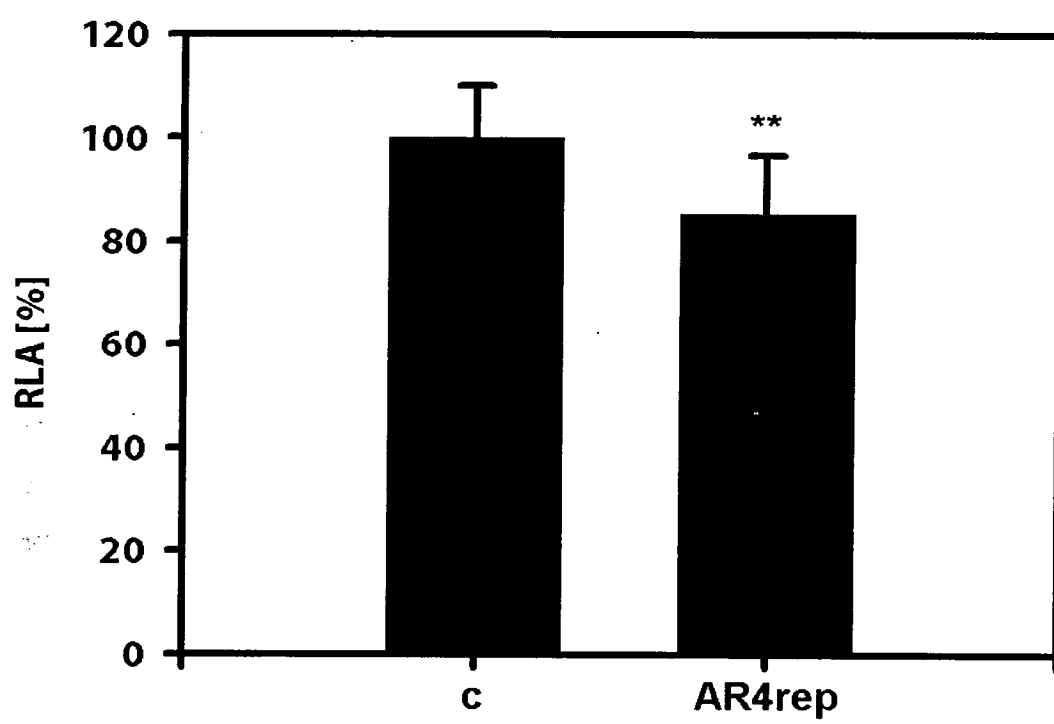


圖5