NURSA
Nuclear Receptor Signaling Atlas

COURSE NOTES

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COURSE: Nuclear Receptor Signaling: Concepts and Models

Publication Date: October 2003

1. Course Information

Transcript

Welcome to "Nuclear Receptor Signaling".

Before beginning, please take a moment to read the course details.
Click on the Course Notes button to download a PDF version of the course. The course notes contain all the text associated with the course.

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Course Details

Course Length: Approximately 2 hours. Your time may vary based on modem speed, prerequisite knowledge and other factors.
Prerequisites: None
Date Published: October 2003

2. Interface Tour

Introduction
It is important that you become familiar with all of the functionalities of this interface so that you can use it optimally to navigate through the course, and to access the transcripts, help and resources sections. This lesson gives you a tour of the interface.

Transcript
Features of this interface include:

The title of the current course.

The title of the current lesson.

Course contents menu:
Click on the section headings to access lessons within that section.

Click on lesson titles to access individual lessons. The current lesson will be highlighted. Completed lessons will be indicated by a different color.

Resources: Click on this heading to access additional information relevant to the current course. The Resources section contains links to other related websites and a complete glossary of terms.

Close: Click on this button to exit the course and close the course window.

Text: Click on this button to access a printable transcript of the voiceover for the current lesson.

Help: At any point in the course, roll your cursor over this button to review the features of the interface.

Click and drag on the playback controller to go to any specific point within the current lesson.
The Previous button takes you to the beginning of the previous lesson.

If you are at the first lesson of the course, this button will be inactive.

The Play/Pause button toggles between play and pause.

The Next button takes you to the beginning of the next lesson.

If you are at the last lesson of the course, this button will be inactive.

At the end of each lesson, the NEXT button will flash, alerting you to go on to the next lesson.
The Play button plays the current lesson.
The Pause button pauses the current lesson.

Information icon: Within certain lessons, you can access more information about the topics covered by rolling your cursor over this icon.

Throughout the course, you will occasionally be able to view additional information by clicking or rolling your cursor over a button on the stage. The voiceover will alert you whenever this occurs.

3. Introduction

Introduction

Nuclear receptors are members of a superfamily of ligand-inducible transcription factors involved in the regulation of specific target genes involved in biological processes as diverse as metabolism, development and reproduction. They mediate the transcriptional response to hormones such as the sex steroids (progestins, estrogens and androgens), adrenal steroids (glucocorticoids and mineralocorticoids), vitamin D_3, thyroid and retinoid (9-*cis* and all-*trans*) hormones, in addition to a variety of metabolic ligands.

A substantial body of work dating from the 1960s to the present day has been devoted to the characterization, cloning, functional analysis and biological exploration of these factors. More recently, the complexity of nuclear receptor signaling pathways has been brought into focus by the discovery of coregulators, a structurally and functionally diverse group of factors which mediate the positive and negative effects of nuclear receptors on gene expression.

This animated course will sketch some of the landmark studies and discoveries in this field since its inception. New sections will be added periodically as the field advances, with key literature references being incorporated as it evolves. While the course is primarily focused at advanced life sciences students and scientists from other disciplines wishing to become acquainted with the field, we anticipate that it will be a useful resource for any user of the NURSA website.

Transcript

The nuclear receptor superfamily is an evolutionarily-related group of transcription factors which regulates genes involved in a variety of physiological, developmental, and metabolic processes.

Members of this superfamily play a role in:

- Regulation of reproductive systems by steroid hormones
- Control of development by thyroid and retinoid hormones
- And regulation of bile acid and cholesterol biosynthesis.

In addition to their normal function, loss of control of nuclear receptor signaling pathways contributes to the development of endocrine-related diseases, such as breast cancer, prostate cancer, ovarian cancer, diabetes and obesity.

Many interesting new facets have appeared in this field in recent years – in this course, we will look at some of these developments, and how they may help to improve therapeutic approaches to a variety of illnesses.

4. Identification and Cloning of Nuclear Receptors

Introduction

The concept of high affinity, tissue-specific, steroidophilic factors - receptors - as mediators of hormone function, gained credence from pioneering tissue-binding studies by Elwood Jensen, Jack Gorski, Tony Means and other pioneers in the field. Experiments defining a chronology for the effects of these molecules in the oviduct, uterus and other target tissues established cell- and ligand-specific increases in mRNA and protein synthesis as primary events in their action, and substantiated a basic linear model of steroid hormone action from ligand to target gene product.

Biochemical purification strategies used radiolabelled ligands to considerable effect to overcome the sparse cellular levels of many nuclear receptors, and by the late 1970s, a steroid receptor, the progesterone receptor, had been purified to homogeneity. Studies using purified receptor fractions, particularly those of progesterone receptor and the glucocorticoid receptor built on the basic model by characterizing receptor dimerization and increased affinity of the receptor for specific *cis* acting DNA sequences - hormone response elements.

Transcript

While steroid hormones had been known to exist since the early 20th century, it was not until the early 1960s that the idea of specific hormone-binding molecules in the target tissues of these hormones began to emerge.

Pioneering studies by Elwood Jensen, Bert O'Malley and others led to the hypothesis that activated receptors were capable of binding to specific DNA sequences in the nuclei of their target cells, and inducing the production of certain proteins in these cells.

By the end of the 1980s, when techniques for cDNA library screening and cloning and sequencing DNAs became routine, the first receptor cDNA, encoding the glucocorticoid receptor, had been cloned in Ron Evans lab in the Salk Institute in La Jolla, California. The cloning of cDNAs encoding receptors for estrogen, progesterone, thyroid hormones, retinoids, vitamin D, aldosterone and testosterone soon followed.

Click on the book icons to access the respective PubMed links.

To read more about this topic, click on the "Further Reading" icon.

5. The Nuclear Receptor Superfamily

Introduction

Building upon biochemical evidence for the DNA binding and hormone binding activities of receptors, comparative alignment of the deduced amino acid sequences of the newly cloned receptor cDNAs indicated a modular organization of discrete motifs in each molecule, indicating a potential common ancestry and mode of action.

Transcript

Comparison of the deduced amino acid sequences encoded by many of the receptor cDNAs showed that they were members of a ligand-activated superfamily of transcription factors — the nuclear receptor superfamily.

Historically, nuclear receptors have been classified as one of two types:

Type I receptors undergo nuclear translocation upon ligand activation and bind as homodimers to inverted repeat DNA half sites, called hormone response elements, or HREs.

This category includes receptors activated by steroid ligands, such as estrogen receptor, progesterone receptor and androgen receptor.

Type II receptors (TR and RAR, among others), are often retained in the target cell nucleus regardless of the presence of ligand, and usually bind as heterodimers with RXR to direct repeats.

Receptors for thyroid hormone, all trans retinoic acid and vitamin D fall into this group.

To read more about this topic, click on the "Further Reading" icon.

6. Functional Domains of Nuclear Receptors

Introduction

While biochemical studies had established DNA sequence binding preferences and other important functional properties of receptors, it was the advent of molecular biology techniques for mutagenesis and modular dissection of receptors that ultimately defined their functional substructure. With the maturation of these techniques to handle much larger sequences of DNA, disruption of the genomic sequences of nuclear receptor genes became possible, allowing the first tangible demonstrations of their diverse biological roles.

Transcript

While analysis of the receptors up to this point had relied largely on biochemical techniques, advances in molecular biology allowed a minute dissection of the functional domains of receptors.

The transient transfection-based HRE-reporter assay , developed by Ron Evans, became the tool of choice for investigating the mechanism by which nuclear receptors regulate their target genes.

In this assay, a cDNA encoding the receptor, or part of a receptor is transfected, into a suitable cell line along with a reporter gene, usually the luciferase gene, linked to a promoter controlled by 1 or more HREs specific for the receptor being studied.

The amount of luciferase enzyme assayed is proportional to the activity of the receptor.

Addition of a specific ligand activates the receptor and touches off a sequence of events, culminating in recruitment of general transcription factors and an increase in the rate of transcription of the reporter gene.

Restriction enzymes were used to mix and match cDNA fragments encoding autonomous functional modules of the receptors, and to generate specific domain and point mutations whose functional effects could be evaluated using the luciferase assay.

When you are finished with this section, click Continue.

In this way, a number of signature domains were identified as hallmarks of many members of this family. The two most important ones are:

- A conserved zinc finger-based DNA binding domain (DBD) which binds hormone response elements , or HREs, in the promoters of target genes; and
- A C-terminal domain, the LBD, containing regions mediating ligand binding and dimerization of compatible receptors

Domains known as activation functions – AF-1 in the N-terminus and AF-2 in the C-terminus - were identified which were of themselves sufficient to activate the luciferase gene in reporter assays.

Also identified were domains that could mediate “silencing” or repression, of a reporter gene.

These results suggested that many nuclear receptors possessed dual functionality, capable of activating transcription of target genes in the presence of ligand or repressing target gene promoters in the absence of ligand. The physiological importance of nuclear receptors has been demonstrated by targeted deletion of their genes in mice.

Deletion of estrogen receptor, progesterone receptor or androgen receptor results in multiple reproductive abnormalities in both males and females.

Deletion of the receptor for 9 cis retinoic acid, RXR, has consequences for embryonic and post-embryonic development in mice.

PPARs and LXRs are major players in glucose & lipid homeostasis

And the glucocorticoid receptor has myriad roles including those in long term spatial memory, stress erythropoiesis and energy metabolism.

To read more about this topic, click on the "Further Reading" icon.

7. Coregulators

Introduction

The ability of nuclear receptors to alternate between activation and repression in response to specific molecular cues, is now known to be attributable in large part to a diverse group of cellular factors, the coregulators. The study of nuclear receptors owed a debt to decades of historical endocrinology and pathology, and prior to their discovery there was a wealth of empirical evidence that suggested their existence. Coregulators, in contrast, have been the subject of a rapid accumulation of functional and mechanistic data which is yet to be consolidated into an integrated picture of their biological functions.

Transcript

In the early 1990s, some investigators such as Keith Yamamoto had suggested a role for non-DNA "nuclear acceptor" molecules. Evidence for the existence of these factors of these factors came from experiments in Pierre Chambon's laboratory in which "squelching", or competition for a common limiting factor, was observed between coexpressed receptors in reporter assays in mammalian cells.

When you are finished with this section, click Continue.

A biochemical strategy designed in Myles Brown's laboratory provided the first direct evidence of ligand-dependent recruitment by nuclear receptors of ancillary molecules.

To read more about this topic, click on the "Further Reading" icon.

8. Cloning and Characterization of Coactivators

Introduction

The functional autonomy of the nuclear receptor ligand binding domain permitted its adaptation to the yeast two-hybrid protein-protein interaction assay, and after pioneering biochemical and genetic screens identifying an array of receptor-interacting factors, the stage was set for the functional characterization of these molecules.

Transcript

To facilitate the cloning of coregulator cDNAs, a molecular screen was designed based upon the yeast two-hybrid protein-protein interaction assay.

First, a hybrid bait molecule was created in which a receptor LBD was linked to the activation domain of a yeast transcription factor, Gal4.

Next, a library containing millions of potential binding partners was created by fusing a human cDNA library with the Gal4 activation domain.

The yeast cells contain a lacZ gene controlled by Gal4 DNA-binding sites. Cells in which the lac Z gene is expressed will turn blue in the presence of a chromogenic substrate, whereas cells in which it is not expressed will remain white in appearance.

In cells in which the bait does not interact specifically with a library protein, expression of the lacZ gene generally does not occur, since the Gal4 transcription factor is not reassembled at the reporter gene promoter.

In cells in which the bait interacts specifically with a library protein, the Gal4 factor is effectively reassembled, and touches off expression of the lacZ gene. These cells appear blue on the plate.

Individual clones are retrieved, their library plasmids recovered, and the cDNAs sequenced to determine their identity.

Proteins were initially designated as coactivators based on their ability to enhance the function of receptors in HRE transient transfection assays, and to relieve squelching between two competing receptors in these assays.

Screens carried out in this way were the first to clone cDNAs encoding nuclear receptor coregulator molecules, the TRIPs in David Moore's laboratory, and RIP140 also known as NRIP1, in Malcolm Parker's laboratory.

The first authentic, common transcriptional coactivator was steroid receptor coactivator 1, or SRC-1, first cloned in Bert O'Malley's laboratory. SRC-1 and two related proteins, GRIP-1, cloned first by Michael Stallcup, and p/CIP, initially identified in Geoff Rosenfeld's lab, make up the SRC/p160 family of coactivators.

The SRC/p160 family is defined by the presence in the N-terminus of tandem PAS and beta-H-L-H motifs;

A centrally-located domain which binds the coactivators CBP and p300;

And a C-terminal region which mediates interaction with the CARM-1 coactivator.

As will be seen later, interactions between coactivators, and the control of these interactions, are thought to be an important component in the diversity of nuclear receptor action.

Malcolm Parker's laboratory was the first to show that a recurring structural feature of many coactivators is an alpha-helical LXXLL motif, or nuclear receptor box, present from a single to several copies in many coactivators, which is implicated in their ligand-dependent recruitment by the receptor AF-2. The SRC/p160 coactivator family, for example, has a conserved cluster of NR boxes located in the central region of each member of the family

Coactivators can be categorized based upon their varied functional properties. Classes of coactivators include:

- Acetyltransferases, such as members of the SRC/p160 family
- Ubiquitin ligases, such as E6-AP
- ATP-coupled chromatin remodeling complexes, such as the SWI/SNF/BRG-1 complex
- Protein methylases, such as CARM-1 and PRMT-1
- RNA transcripts, such as SRA
- Cell cycle regulators such as cdc 25B
- RNA helicases such as p72
- And members of the TRAP/DRIP complex, which foster direct contact with components of the basal transcription machinery

Click on the book icons to access the respective PubMed links.

To read more about this topic, click on the "Further Reading" icon.

9. Corepressors

Introduction

Transcriptional repression by corepressors is in many ways conceptually comparable to the mediation of receptor transcriptional activation by coactivators. Their recruitment of corepressors, generally occurring in the absence of ligand, depends on a critical conformation of the receptor AF-2 domain, as well as upon nuclear receptor box-like helical motifs in the corepressor. Moreover, corepressors themselves recruit ancillary enzyme activities which help to establish or maintain the repressive state at their target promoters.

Transcript

Experiments similar in design to the coactivator yeast two-hybrid protocols were used to identify molecules which mediated the repressive functions of nuclear receptors.

Early cell transfection experiments had shown that discrete regions of certain receptors, such as thyroid hormone receptor, were sufficient to repress, or silence, reporter genes when fused to DNA-binding domains of heterologous transcription factors, suggesting that specific cellular factors – or corepressors - might bind to these regions.

Using the yeast two-hybrid screen, two corepressors were isolated in rapid succession, nuclear receptor corepressor, or in Geoff Rosenfeld's laboratory, and silencing mediator of retinoid and thyroid receptors, or SMRT, by Ron Evans. Alignment of the two proteins indicated that they had a largely common domain structure, suggesting parallels in their mode of action.

Mitch Lazar's group has shown that inactive nuclear receptors recruit corepressors in part through amphipathic helical peptides called CoRNR boxes, which are similar to the coactivator nuclear receptor boxes.

In addition to these structural analogies, corepressors and coactivators have common functional themes. The acetylation state of nucleosomes on a promoter is related to the rate of transcription of the gene. Histone acetylase coactivators increase the rate of acetylation, opening the nucleosome to transcription factors; histone deacetylases recruited by corepressors reverse this reaction, silencing transcription of the target gene.

To read more about this topic, click on the "Further Reading" icon.

10. Coregulators *in vivo*

Introduction

The physiological role of SRC/p160s, CBP/p300 and other coactivators has been implied by knockout studies in mice of genes encoding these proteins. The effects of these deletions range from the profound effects on viability characteristic of TRAP220, CBP and p300, to the more subtle developmental and metabolic phenotypes associated with members of the SRC/0160 family.

Transcript

Using sequences from cloned coregulator genes, laboratories such as those led by Bert O'Malley, Bob Roeder, Geoff Rosenfeld and Pierre Chambon were able to delete, or knockout these genes in mice. These studies showed that coactivators were required for physiological and developmental functions of steroid and thyroid hormones in living animals, and that corepressors too have crucial roles in the development of certain organs.

Click on the book icons to access the respective PubMed links. When you are finished with this section, click Continue to learn more about targeted gene deletion or click the Next button to go on to the next lesson.

To create "knockout" mice, a construct containing the inactivated test gene is first created. This construct is introduced into Embryonic Stem or ES cells. The construct aligns with and hybridizes to the homologous gene; or it hybridizes randomly with other genes within the cell DNA. The small percentage of cells that grow are expanded and injected into a blastocyst, which is then implanted into a pseudopregnant mother. Since the injected cells and the blastocysts are usually derived from different-colored strains of mice, the resulting progeny have coats of two different colors. These mice are inter-crossed to obtain mice lacking an active gene on both chromosomes. The resulting mice can then be studied to determine the function of the missing gene.

11. Regulating the Coregulators

Introduction

A spectrum of post-translational modifications is known to regulate the functional relationships between nuclear receptors, their coregulator complexes, and their target gene networks. Targeted, reversible enzymatic modifications such as acetylation, methylation, phosphorylation and terminal modifications such as ubiquitination have been shown to have a variety of effects on coregulator function.

Transcript

Coregulators are modulated by a variety of posttranslational modifications, which have specific effects on their function.

Some of these, such as acetylation and ubiquitination appear to alter the half-life of molecules in coactivator complexes.

Methylation may potentiate coregulator function by targeting histones to complement the activity of other coregulators.

Recent work suggests that coregulators may serve to integrate nuclear receptor signaling with inputs from a variety of other signal pathways, such as those involving MAP kinases, protein kinase A, and stress-activated pathways. Phosphorylation of coactivators by cellular kinases has a variety of effects:

- It can specify the movement of coactivators from one compartment to another within the cell
- It can enhance coregulator enzymatic activities such as acetylation
- It can promote the recruitment by one coactivator of another
- And phosphorylation cascades can signal the disruption of inhibitory coregulator complexes

Coregulators may be viewed as control interfaces for integrating multiple afferent stimuli into an appropriate cellular response. One possible scenario is that differential phosphorylation of coactivators may direct their combinatorial recruitment into different transcriptional complexes at distinct promoters.

To read more about this topic, click on the "Further Reading" icon.

12. Nuclear Receptor Action Model

Introduction

Coactivators are thought to exist in large, modular complexes in the cell, and are known to participate in many different protein-protein interactions. A current model is that the composition of these complexes can become fluid, mixing and matching subunits to tailor the specific needs of different receptors, ligands or promoters.

While spatiotemporal aspects of nuclear receptor and coregulator action remain poorly defined, a broad composite model of nuclear receptor action invokes corepressors as critical mediators of nuclear receptor silencing. In turn, a variety of coactivators are implicated in transcriptional activation by nuclear receptors, including SWI/SNF chromatin remodeling machines, SRC/p160s and TRAP/DRIP. The model accommodates the ability of G protein coupled signaling pathways to cross-talk with coactivator and corepressors function at the transcriptional level.

Transcript

In a current consensus model of nuclear receptor action, initial binding of ligand to the receptor results in dissociation of corepressors and recruitment of SWI/SNF chromatin remodeling machines to modify chromatin domains.

Binding of SRC/p160s and CBP results in local acetyltransferase activity and disruption of local nucleosomal structure.

Kinase-mediated signaling pathways may communicate directly with nuclear receptor-regulated promoters. AF-1 phosphorylation might serve to further consolidate ligand-dependent NR-SRC/p160 interactions, or to recruit SRC/p160s directly to receptor in the absence of ligand.

TRAP/DRIP directly contacts components of the basal transcription machinery to effect transcriptional initiation, and certain TAFs may afford some additional input into promoter-specific nuclear receptor transcription.

To read more about this topic, click on the "Further Reading" icon.

Click here to review selected classes of coactivators.

Click on the book icons to access the respective PubMed links.

13. Rational Drug Design

Introduction

The wealth of molecular data accumulated to date on functional interactions between nuclear receptors and coregulators has exciting implications for the development of novel pharmaceutical therapies for a wide range of diseases. Clinical strategies addressing the role of coactivators as platforms for integrating stimuli for cell proliferation with steroid receptors are likely to meet with success in the treatment of breast cancer and other reproductive cancers. Moreover the functional importance of coregulators in signaling by receptors involved in energy metabolism suggests that probing this aspect of their action may prove to be a rewarding avenue of research.

Transcript

Evidence implicates steroid hormones in a variety of neoplastic diseases such as breast cancer, ovarian cancer and prostate cancer.

The discover that AIB1 is overexpressed in up to 60% of primary breast tumors and in 15% of primary ovarian tumors suggested that high cellular levels of this factor might potentiate estrogen action in these tumors through increased interaction with the estrogen receptor.

The interface between receptor AF-2 elements and the coregulator nuclear receptor box has been the subject of intense study as a potentially rewarding target for peptide-based manipulation of nuclear receptor pharmacology.

Crystallography of the AF-2 NR box interface has identified a ligand-induced hydrophobic cleft as a principal determinant of the interaction of AF-2 with the nuclear receptor box.

The design of peptidomimetics to mimic the structure of the coregulator nuclear receptor box may have considerable potential in uncoupling the ER-coactivator pathway in breast cancer and in other diseases.

To read more about this topic, click on the "Further Reading" icon.

14. Self Test Quiz

Introduction
Congratulations! You have reached the end of this course. To complete the course, please proceed to the Self Test Quiz.

You may take the Self Test Quiz now, or later at your own convenience. Before beginning the quiz, please feel free to review the lessons as often as required.

You will be presented with questions one at a time. You may move back and forth between the questions to revise your answers if necessary.

When you are satisfied with your answers, you can submit them to be graded.

Transcript
Congratulations! You have reached the end of this course. To complete the course, please proceed to the Self Test Quiz.