

Topical Review:**Formation of a national research effort in muscle growth and development in animal sciences: Project NC-131**Brad Creamer¹, J.L. Vierck¹, L. Miller² and M.V. Dodson^{1*}¹*Muscle Biology Laboratory,
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From an original focus on whole animal or carcass research, the field of classical meat science has evolved into a number of modern disciplines. One of these disciplines, muscle growth biology, utilizes biochemical, cellular or molecular approaches to increase lean muscle growth in animals without sacrificing the quality of the final meat product. A group of nontraditional animal scientists envisioned that change was needed and facilitated this evolution of muscle growth biology away from meat science. Through the years, the discipline has been fostered by a strong group of mentors, most of who are descendents of the original nucleus of forward-looking scientists. This mentoring group collectively came together under the auspices of an USDA-sponsored research project, North Central number 131 (NC-131), entitled: "Molecular mechanisms regulating skeletal muscle growth and development." The student conceived this paper as an undergraduate research project, with the intent to learn of the establishment, rich history and evolution of a portion of the field he was thinking of entering to obtain an advanced degree following graduation.

Introduction

As the field of Animal Science progresses into the 21st Century, it seems appropriate to reflect on significant achievements of the past. Great strides have been made in many of the areas encompassed by Animal Science, such as improvements in management, nutrition, genetic selection and reproduction. Research efforts within these disciplines have led to the discovery of new and fundamental information about the molecular and cellular processes that regulate animal growth and development of skeletal muscle. Many of the improvements and successes in the area of meat production have been accomplished through the efforts of the members of the multi-state, regional research project, NC-131, "Molecular mechanisms regulating skeletal muscle growth and development." For the past 25 years, NC-131 scientists have been studying ways to increase lean muscle growth in food producing animals, resulting in many achievements in the field of meat science. As new members step into the roles of mentors, professors, and scientists, we

hope that an appreciation and understanding of the work of those who preceded them will provide a standard of excellence and a strong base of knowledge that will be passed along to future generations of Animal Scientists.

Overview of NC-131 Research

Previous research involving genetic selection and improved nutrition for livestock animals contributed to an increase in skeletal muscle growth. As practices became optimized, however, it became apparent that additional information about the molecular mechanisms and specific genes that control skeletal muscle growth was needed. To address this lack of knowledge, the NC-131 regional research project was formally initiated in 1975 and has been renewed five times since then. The major emphasis of this group has been on basic research, with minor objectives specifically directed toward finding ways to increase the efficiency of lean meat production by food-producing animals. Important discoveries regarding calpains, general protein synthesis and

accumulation, myofibrillogenesis, satellite cells, and hormones and growth factors have been made and documented by this group.

The original objectives of the NC-131 research project were clearly defined. The first objective was “Specify the factors that regulate the rate of protein synthesis and assembly of synthesized proteins into functional structures in muscle cells”. Because of the nutritional importance of myofibrillar proteins and their involvement in meat tenderness, preliminary efforts were directed toward the study of the factors regulating their synthesis and assembly. The second objective was “Identify the factors that regulate the rate of disassembly of functional protein structures and degradation of disassembled proteins in muscle cells”. Research addressing this objective investigated the role and regulation of muscle proteases on the initiation of myofibrillar breakdown (turnover); myofibrillar, soluble, and total protein degradation in individual muscles and muscle cell cultures; and total body protein turnover. The nature and location of any enzymes involved in proteolysis were also studied. The third objective was “Determine the relationship of number, size and metabolic nature of cells in muscle tissue to muscle growth”. Research resulting from this objective studied the relationship between prenatal and postnatal muscle fiber development, the relationship between satellite cell number and proliferative activity, and the mechanisms of hormone action in stimulating myogenic cell proliferation. Animal models were selected for the three objectives so that data generated by all participating stations could be compared. This resulted in an integrated collection of findings that were more complete and informative than could be obtained by any single station working alone. The objectives of the NC-131 regional research project remained constant from 1975-1985.

In 1985, the NC-131 committee changed two of the three original research objectives. The first objective remained the same, but the second and third objectives were modified. The decision to change direction with the second objective was made because a majority of the studies looking at the calcium-activated protease in muscle were completed. This protease had been given several names: calcium-activated factor (CAF), calcium-dependent protease (CDP), and calpain. Consensus of the group was that future work in this area could be combined under

the umbrella of objective one. Therefore a new second objective was written: “Determine the hormonal, metabolic, and nutritional factors that regulate myogenic cell proliferation, differentiation, and maturation”. This objective focused on research in four areas: The characterization of the relationship between myonuclei number and muscle growth in animals; the identification of the factors that regulated myogenic cell proliferation, differentiation and maturation; the determination of the specific mechanisms involved in the regulation of myogenic cell proliferation, differentiation and maturation; and the examination of the regulation of muscle maturation and senescence. The third original research objective was changed to: “Develop applications of modern techniques in molecular biology, genetic engineering and hybridoma technology for use both as research tools for the study of muscle cell growth and as methods for modifying muscle cell growth in meat animals”. Two specific areas included within this new objective were the applications of recombinant DNA techniques to study muscle growth and the production of monoclonal antibodies.

In the 1990 project revision, the third objective was again changed: “Characterize the effects and mode of action of hormones and growth factors on growth of muscle tissue”. This objective was aimed toward understanding how specific regulatory factors direct the overall process of muscle growth. It was proposed that the information obtained from previous objectives concerning molecular mechanisms of growth regulation could be applied to culture systems and animal models for the development of new methods to increase the efficiency of muscle deposition in meat animals. In the 1995-2000 revision, the objectives were again modified to reflect an emphasis on molecular mechanisms, while remaining broadly defined enough to allow for changes in the direction of the work during the five-year period. The first objective was “Characterize the signal transduction pathways that regulate skeletal muscle growth and differentiation”. Three specific areas of research were proposed to address this objective. The first area involved the characterization of the function and expression of the growth factors, receptors and binding proteins involved in the signaling pathways that influence skeletal muscle development. The second area investigated the role of the extracellular matrix in signal transduction

pathways in developing muscle. The third area concentrated on elucidating the intracellular pathways that transmit receptor signals to the nuclei of myogenic cells.

The second objective was also modified to “*Determine the nuclear mechanisms that control gene expression in skeletal muscle.*” Two specific research foci were proposed to address this objective. The first plan was to isolate and sequence muscle protein genes and map their promoter/enhancer regions. The second was to identify and characterize transcription factors that regulate important skeletal muscle genes.

The third objective of the 1995-2000 proposal was “*Characterize muscle proteins and their functional domains involved in myofibrillar assembly and disassembly.*” This area of study was divided into two subdivisions: to define the mechanisms of myofibrillar protein assembly, and to determine the mechanisms regulating disassembly and degradation of myofibrils.

Through the years the overall research goal of the NC-131 group has been to increase the efficiency of lean meat production in domestic, food-producing animals. A common theme of the work performed by this group is that methods for improving the efficiency of lean meat production and enhancing the quality of the final product depend on fundamental knowledge of the underlying mechanisms that regulate growth of skeletal muscle tissue and cells in animals. As knowledge and information have been collected through the research efforts of NC-131 committee members, the objectives of the collective research have changed, but the basic fundamentals have remained constant.

History of NC-131; How did it get started?

During the 1973 annual meetings of the American Society of Animal Science (ASAS) held at Lincoln, Nebraska, a group of North Central (NC) researchers, along with several other interested people from outside the geographic region, discussed the concept of devising a regional research project focusing on muscle growth and development. It was not until August of 1973, however, that the formal process of establishing a North Central Technical (NCT) committee was attempted. Formation of a recognized NCT

committee was the first step in obtaining the official stamp of approval for the research project. In order to be approved as an NCT committee, an initial proposal was required from interested participants. Therefore, a total of 31 researchers from the NC region of Texas, California and USDA Beltsville were asked for input on the project and if they were interested in becoming official participants in writing the NCT proposal. Twelve researchers from four research stations agreed to help (Table 1), with Dr.’s Darrel Goll and Gene Allen providing the leadership.

NCT-109 Station/Member	January 16 Station/Member
Minnesota E. Allen	California C. Ashmore
P. Addis V. Hegarty	Indiana E. Aberle
Michigan R. Merkel W. Bergen	Iowa D. Goll F. Parrish R. Robson M. Stromer
Purdue E. Aberle	D. Topel A. Trenkle
Iowa F. Parrish D. Topel R. Robson M. Stromer A. Trenkle D. Goll	Michigan W. Bergen R. Merkel R. Young
	Minnesota P. Addis W. Dayton V. Hegarty
	Missouri M. Bailey
	Pennsylvania R. Martin
	Texas T. Dutson
	Wisconsin R. Bray M. Greaser
	Clay Center R. Prior
	CSRS S. Zobrisky

Table 1. Original NCT-109 committee stations and members, and NC-131 stations and members at the January 16, 1976 meeting.

The proposal to form an NCT committee was submitted on December 3, 1973, and was subsequently reviewed and approved by the Research Committee of the NC Directors' Association. NCT-109, entitled "Protein synthesis and growth of skeletal muscle," was officially recognized in April 1974. Establishment of the recognized NCT-109 committee was the first step in getting approval for a regional research project sponsored by the USDA-Cooperative State Research Service (CSRS). The next goal of this group was to write a full-length proposal, outlining the entire scope and research specifics of the project, which was due by the end of January 1975. By dividing the writing effort among teams, it was thought that a preliminary draft could be finished by November 1974. Dr.'s Parrish and Topel were asked to write the Justification section, while Dr.'s Goll, Robson, Trenkle, Dayton and Young prepared the Procedures and Previous Work sections. After completing a preliminary draft, the NCT-109 committee met at Iowa State University in January 1975 to finalize the proposal.

By February 18, 1975, the final version of the proposal for the establishment and funding of the regional NCT-109 committee was submitted. This proposal was built around fundamental "approaches toward determining how the factors regulating muscle protein synthesis and degradation regulate the rate and extent of muscle growth in meat animals." The project, now entitled "Factors regulating rate of protein

synthesis and growth in skeletal muscle," had two applied goals: to increase feed efficiency in meat animals and to discover biochemical factors which limit muscle growth. In May 1975, Dr. Robert Bray, Administrative Advisor of the USDA-CSRS, notified Dr. Goll of the official approval of the proposal, with a new designation of NC-131 and a duration of five years.

The first official meeting of the NC-131 regional research committee was held at the University of Missouri on June 18, 1975, in conjunction with the Reciprocal Meats Conference. In this brief organizational meeting, officers were elected: Dr. Goll, chairman; Dr. Ashmore, vice chairman; and Dr. Dutson, secretary. It was decided to hold the next scientific meeting of the NC-131 committee at the academic home of the chairman in less than six months. Interestingly, during the six-month interval between the organizational meeting and the first regular scientific meeting of the NC-131 group, many newly interested scientists petitioned to join the NC-131 committee. By the January 1976 meeting the NC-131 committee grew to 21 members (Table 1).

NC-131 Structure

Because of the cooperative nature established by the members of the NC-131 regional committee, there have been numerous member stations (Figure 1) throughout

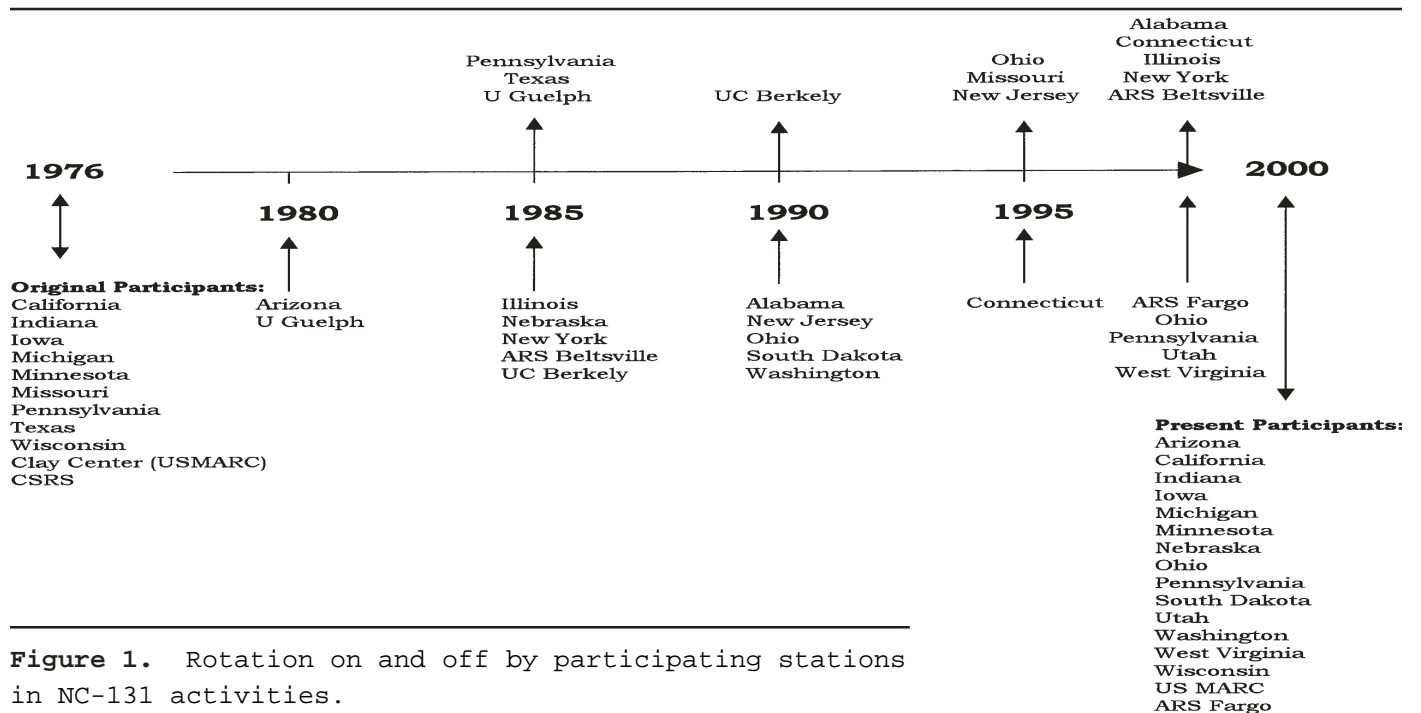


Figure 1. Rotation on and off by participating stations in NC-131 activities.

the twenty-five year history of the group. When the project officially began in 1976, there were eleven participating stations. When the most recent renewal of the NC-131 project ended in 2000, there were fourteen participating stations, as well as two USDA laboratories. Six of the original member stations are still actively involved in the NC-131 project. In order for a station, agency, or institution to become a participant in the project, they must petition to join by submitting a proposal describing their work and how it will contribute to the committee. The NC-131 technical committee then reviews it for consideration and approval. To remain a member of the project, each unit must present both an oral and a written report of its research results at a yearly meeting of the technical committee. These reports are critically reviewed, and then recommendations are made for future research projects. Any unit that does not submit a written progress report for two consecutive years is considered to be a non-participating station and may be dropped from the project. A list of the NC-131 participants over 25 years is shown in Table 2.

Accomplishments of NC-131

From 1975 to 1999, a total of 57 M.S. and 62 Ph.D. candidates received degrees under the guidance of NC-131 members, suggesting that the research and knowledge gained from mentoring students during the past twenty-five years has added to the accomplishments of this committee. An abbreviated version of a genealogical record of one of the original members of this research group, Dr. Darrel Goll, is shown in Figure 2. All the people listed have participated in NC-131-related research and are currently training students in the field of muscle growth and development. This research has led to many publications and awards for the committee. From 1975 to the present, NC-131 scientists have published approximately 800 scientific papers, book chapters and proceedings' articles and 737 abstracts (Figure 3, p. 14). While it would be impossible to list all of the accomplishments of the committee, highlights and descriptions of some of the research from NC-131 participants follow.

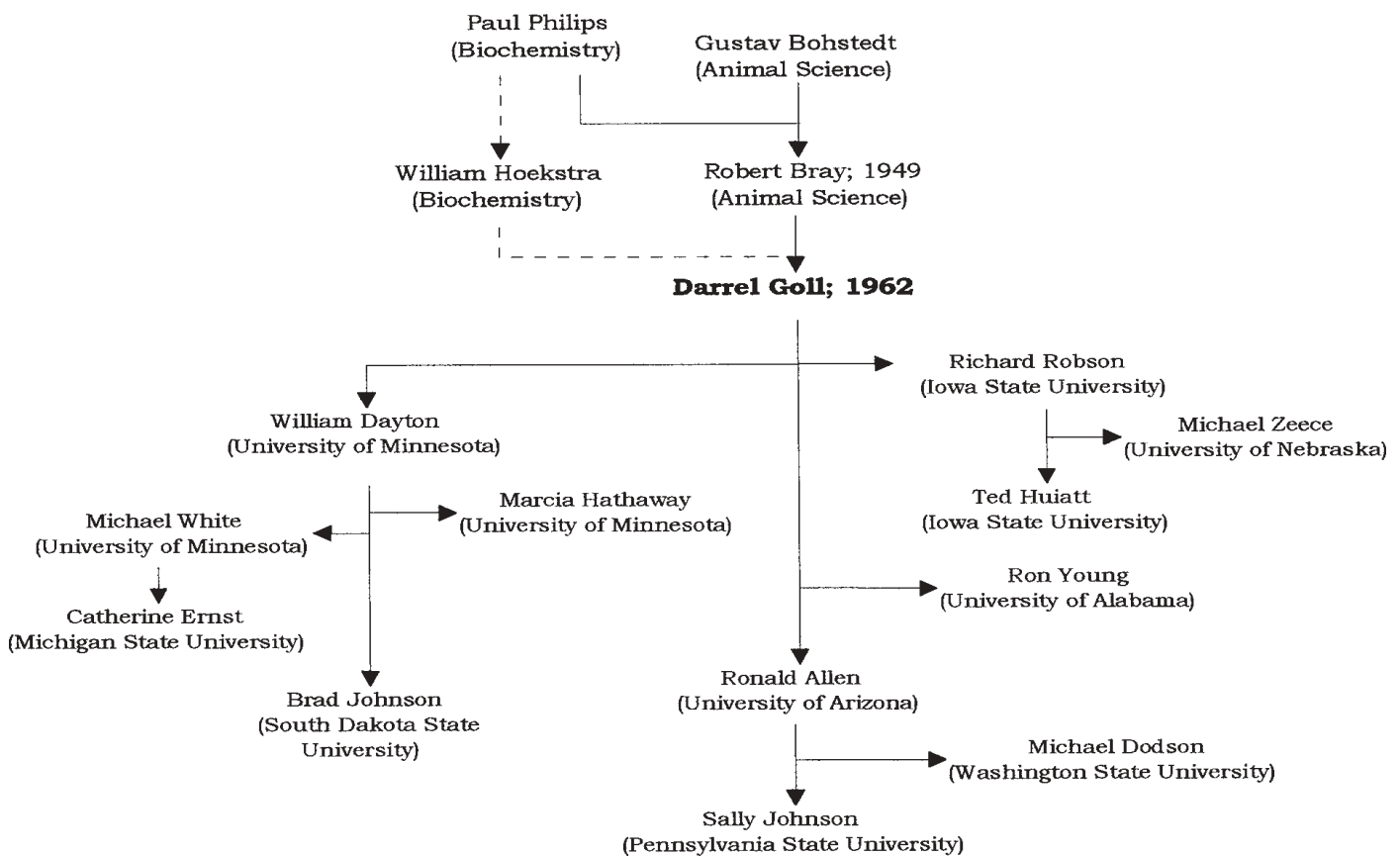


Figure 2. Genealogy of Dr. Darrel Goll, a founder of the NC-131 group, showing student continuity.

<u>Participating State/Agency</u>	<u>Member</u>	<u>Area of Specialization</u>
Alabama	Davenport, G.	Nutrition
	Mulvaney, D.	Cell Biology
Arizona	Allen, R.	Biochemistry/Cell Biology
	Goll, D.	Biochemistry
California	Ashmore, C.	Meat Science
	Bandman, E.	Biochemistry/Cell Biology
	Lee, Y-B.	Muscle Biology
	Richmond, J.	Biochemistry
Illinois	Bechtel, P.	Biochemistry
	McCusker, R.	Endocrinology/Cell Biology
Indiana	Aberle, E.	Meat Science
	Grant, A.	Molecular Biology
	Gerrard, D.	Cell Biology
	Handcock, D.	Molecular Biology/Nutrition
	Mills, S.	Biochemistry
Iowa	Orcutt, M.	Meat Science/Cell Biology
	Goll, D.	Biochemistry
	Huiatt, T.	Biochemistry
	Nissen, S.	Nutrition/Endocrinology
	Reecy, J.	Molecular Biology
	Robson, R.	Biochemistry
	Stromer, M.	Molecular Biology
	Topel, D.	Meat Science
Michigan	Trenkle, A.	Nutrition
	Bergen, W.	Nutrition
	Doumit, M.	Cell Biology
	Ernst, C.	Molecular Biology
	Helferich, W.	Molecular Biology
	Merkel, R.	Meat Science
	Young, R.	Molecular Biology
Minnesota	Addis, P.	Muscle Biology
	Allen, C.	Meat Science
	Dayton, W.	Biochemistry/Cell Biology
	Hathaway, M.	Cell-Molecular Biology
	Hegarty, V.	Muscle Biology
Missouri	White, M.	Molecular Biology
	Bailey, M.	Biochemistry
Nebraska	Jones, S.	Biochemistry/Meat Science
	Zeece, M.	Biochemistry/Meat Science
New Jersey	Fagan, J.	Biochemistry
New York	Beerman, D.	Physiology/Anatomy
Ohio	Ramsay, T.	Physiology
Pennsylvania	Johnson, S.	Molecular Biology
	Wagness, P.	Nutrition
South Dakota	Johnson, B.	Extension
	McFarland, D.	Cell Biology
Texas	Dutson, T.	Meat Chemistry
	Landmann, W.	Biochemistry
Utah	Carpenter, C.	Biochemistry
Washington	Byrne, K.	Molecular Biology
	Dodson, M.	Cell Biology
West Virginia	Killefer, J.	Molecular Biology
Wisconsin	Greaser, M.	Biochemistry
	Koohmaraie, M.	Meat Science/Cell Biology
ARS/USDA	Mitchell, A.	Nutrition
	Maruyama, K.	Biochemistry/Physiology
	Schollmeyer, J.	Cell Biology
	Shappell, N.	Physiology/Cell Biology

Table 2. State, Participant & Area of Specialization in NC-131 activities (1975-2000).

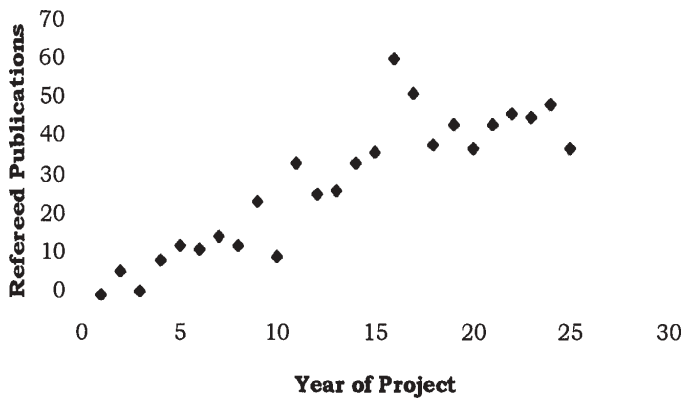


Figure 3. Depiction of productivity of NC-131 members, in which total publications per year are compiled.

Calpains

One focus of the NC-131 committee has been to identify agents responsible for the degradation of muscle proteins and the mechanism(s) by which intact myofibrils are disassembled and the myofibrillar proteins are degraded. NC-131 scientists were able to isolate and purify a calcium-activated protease from muscle and determine that it was an important agent in the metabolic degradation of myofibrils (Dayton, et al., 1974) and possibly in the degradation of z-disk proteins (Dayton, et al., 1975, 1976a,b). Eventually, the protease was given several names, including calcium-activated factor (CAF), calcium-dependent protease (CDP), and calpain (Goll, et al., 1998). Calpain has been isolated from a number of different types of cells and shown to exist in two forms. The first form requires millimolar concentrations of calcium (m-calpain) for activation, while the second needs micromolar concentrations of calcium (μ -calpain). Both polypeptides of calpain are phosphorylated at multiple sites (Huff-Lonergan, et al., 1996). The calpain system plays a variety of roles in transmitting intracellular signals to the nucleus and participates in an integrin-mediated signal transduction pathway (Goll, et al., 1998). Calpains also selectively cleave many of the proteins involved in the receptor/cytoskeleton interaction (Goll, et al., 1999) and have been shown to degrade tropomyosin, troponin T, troponin I, C-protein, filamin, desmin, vinculin, titin and nebulin (Dayton, et al., 1980; 1981a,b; 1982). A new calpain was recently proposed using mRNA encoding techniques and was named skm-calpain (skeletal muscle-calpain), because it was

expressed exclusively in skeletal muscle. Considerable effort is currently being given to the isolation of the protein encoded by the skm-calpain gene. A more complete understanding of calpains will aid in future research on meat tenderness and myofibrillar protein breakdown.

Protein Synthesis and Myofibrillogenesis

Another goal of the NC-131 committee has been to decipher the regulation of the rate of protein synthesis and the accumulation of synthesized proteins in muscle cells. Various approaches have been used to achieve this end. One initial attempt was to develop a cell-free protein synthesis system. A protocol for cell-free RNA synthesis was developed and used to study transcription and translation of myosin and actin mRNA (Allen, et al., 1979). Several studies were also undertaken to determine factors influencing the recruitment of ribosomes to polysomes. Serum deprivation decreased the proportion of ribosomes in the form of polysomes in both myoblasts and myotubes, whereas the addition of insulin increased the proportion of ribosomes in the polysome fraction. In part by using *in vitro* binding techniques (Tan, et al., 1993), it was shown that the myofibrillar proteins actin, myosin, tropomyosin and α -actinin were synthesized in a coordinate manner (Allen, et al., 1979). By characterizing the biochemical properties of the myofibrillar/cytoskeletal proteins, conditions necessary for myofibrillogenesis *in vitro* were discovered (Huiatt, et al., 1980; O'Shea, et al., 1981; Robson, et al., 1981). It was learned that desmin functioned in the cell to connect adjacent myofibrils at the level of the z-line and to maintain the integrity of the muscle cell (Robson, et al., 1984). Vinculin was found to facilitate the interactions between myofibrils and the cell membrane (Robson, et al., 1984). Titin is a very large polypeptide and was found to have a role in the organization of thick and thin filaments during development of the myofiber (Pan, et al., 1994). Talin is a polypeptide located in cellular adhesion plaques and was determined to connect the intracellular cytoskeleton, including the myofibrils, to extracellular receptors (Zhang, et al., 1996). Understanding the mechanisms and mode of association and formation of the myofibrils has been a major area of study for the NC-131 committee.

Satellite Cells

When the committee began in 1975, little was known about satellite cells. Although it was evident that these cells could divide, and that some of the progeny of the daughter cells were incorporated into muscle cells, the origin and physiological role of satellite cells remained unclear. An important advancement emanating from this research area has been the identification of hepatocyte growth factor (HGF) as the critical element in the activation of quiescent satellite cells in postnatal skeletal muscle (Allen, et al., 1995; Tatsumi, et. al, 1998). For years, investigators have searched for the factor or factors responsible for stimulating satellite cells to initiate proliferation and eventually to incorporate into adult muscle fibers, which is an essential part of postnatal muscle growth. Cultured satellite cells themselves synthesize and secrete HGF, indicating that satellite cells can be activated via an autocrine as well as a paracrine mechanism. Satellite cell physiology is still not fully understood. However, with the more recent isolation of satellite cells from sheep, equine, canine, trout, and elk muscle (Greene, et al., 1995; Greenlee, et al., 1995; Vierck, et al., 1995), these cells continue to be a highly researched area of muscle biology.

Other Hormones and Growth Factors

The prime objectives of research in this area have been to develop agents that stimulate skeletal muscle protein accretion and depress total body fat accumulation and to characterize the mode of action of these compounds. Agents such as growth hormone, β -adrenergic agonists, and steroid implants (estrogenic and androgenic) have all promoted live-weight gain of food-producing animals to varying degrees. Combinations of experiments using muscle cell cultures, isolated muscles, and whole animal models have been used to determine the quantitative effects of hormones, growth factors, and other compounds, such as β -agonists, on muscle growth and protein accumulation in muscle tissue (Pringle, et al., 1993). At the cell level, many different types of growth factors are being researched, such as insulin-like growth factor and fibroblast growth factors, both of which influence the rate of satellite cell proliferation

(Sheehan and Allen, 1999). Many of the hormones and growth factors studied have been shown to work in conjunction with other factors to regulate protein synthesis, accumulation, degradation and muscle cell growth.

Implications of NC-131

The NC-131 research project was founded twenty-five years ago in order to initiate a national effort to research the regulation of protein synthesis and the development and growth of skeletal muscle. Through the cooperative efforts of regional stations and USDA laboratories and through the sharing of techniques, culture models, antibodies, molecular probes, and expertise, the NC-131 project continues to benefit both producers of meat animals and researchers in muscle biology. The substantial amount of information provided by this committee about muscle growth in meat animals has proven to be beneficial to all involved in Animal Science. With innovative new technology becoming more widely available, and with the ongoing dedicated work of the NC-131 members, the growth, development and quality of meat products will continue to be enhanced.

Acknowledgements

This research was conducted by Bradley Creamer as an undergraduate student enrolled in A.S. 499 (Directed Research Problems) at Washington State University. Bradley Creamer is presently pursuing a M.S. degree in muscle biology (in Animal Sciences) at the University of Nebraska.

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